Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.


# A chemical approach for the synthesis of the DNA-binding domain of the oncoprotein MYC

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## 1. CONTENTS

## 2. ABBREVIATIONS AND STRUCTURES

Boc: tert-butoxycarbonyl

Dbz: 4-(methylamino)benzoic acid

DEDTC: sodium diethyldithiocarbamate

DIC: *N, N*-diisopropylcarbodiimide

DIEA: *N, N*-diisopropylethylamine

DMEM: Dulbecco's Modified Eagle's Medium

EDTA: ethylenediaminetetraacetic acid

Fmoc: 9-fluorenylmethoxycarbonyl

Fmoc-Ava-OH: 5-(Fmoc-amino) valeric acid

Gn·HCl: guanidine hydrochloride

HATU: 2-(1H-7-aza-benzotriazol-1-yl)-1,1,3,3-tetramethyluroniumhexafluorophosphate

MPAA: (4-carboxymethyl) thiophenol

MS. Mass spectrometry

Nbz: *N*-acyl-*N*'-methylacylurea of Dbz

OSu: *N*-hydroxysuccinimide ester

PBS: phosphate-buffered saline

PEG: polyethylene glycol

TFA: trifluoroacetic acid

Thz: 1,3-thiazolidine-4-carboxo

TAMRA: tetramethylrhodamine

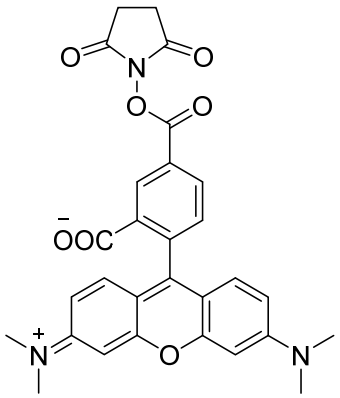
TCEP: tris(2-carboxyethyl) phosphine

TIS: triisopropylsilane

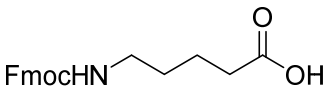
TMEDA: *N, N, N', N'*-tetramethylethylendiamine

VA-044: 2-2'-Azobis[2-(2-imidazolin-2-*yl*) propane]dihydrochloride

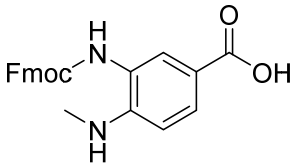
TAMRA-OSu:



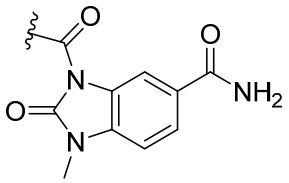
Fmoc-Ava-OH:



Fmoc-Dbz-OH:



Nbz



## 3. REAGENTS AND MATERIALS

Reagents were purchased from commercial sources and used without further purification: Standard Fmoc amino acids, Boc-Ala, *N*-[(*1H*-benzotriazol-1-*yl*)(dimethylamino) methylene]-N-methylmethanaminiumhexafluorophosphate (HBTU) and *N*-[(dimethylamine)-1*H*-1, 2, 3- tiazolo [4, 5- b]piridin-1-il-metilen]-*N*-methyl methanaminium hexafluorophosphate (HATU) from *GL Biochem (Shanghai)*, and Boc-L-thiazolidine-4-carboxylic acid from *Bachem*. TIS (triisopropilsilane), EDT (ethanedithiol), Tris(2-carboxyethyl) phosphine hydrochloride (TCEP•HCl), 4-mercaptophenilacetic acid (MPAA), *N,N*-dimethylformamide (DMF) and acetonitrile ($CH_3CN$) were purchased from *Sigma-Aldrich*. *N, N*-Diisopropilethylamine (DIEA), dichloromethane and trifluoroacetic acid (TFA) were purchased from *Fischer Scientific UK*. 2-2'-Azobis[2-(2-imidazolin-2-*yl*) propane] dihydrochloride (VA-044) was purchased from *Wako Pure Chemical Industries*. All other reagents were purchased from sigma Aldrich.

For ligation experiments, Guanidine Hydrochloride Ultrapure (Gn·HCl, *MP Biomedicals*) was used.

For EMSA experiments, BSA was purchased from *Promega Biotech*, the Nonidet P-40 from *Roche diagnostics GmbH*, the 30% acrylamide: bisacrylamide solution from *Bio-Rad Laboratories, Inc.* and glycerol from *Fluka*.

Peptides were synthesized on Rink-amide-ChemMatrix resin (0.57 mmol/g loading) from *Biotage AB*, or on PAL-PEG-PS resin (0.19mmol/g loading) from *Applied Biosystems*, depending on the case.

For Fmoc-Dbz-OH synthesis we used: 4-Fluoro-3-methylbenzoic acid, methylamine solution, palladium on carbon and 9-fluorenylmethyl *N*-succinimidyl carbonate (Fmoc-OSu). All of them were purchased from *Sigma Aldrich*.

## 4. NMR, HPLC, MASS SPECTROMETRY, UV AND CD MEASUREMENTS.

### 4.1. Reverse phase high performance liquid chromatography (RP- HPLC)

Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) was performed using an *Agilent 1100 Series* Liquid Chromatograph system. Analytical HPLC was run using a *Jupiter C4 column* (5 µm, 300 Å, 150 × 4.60 mm) at a flow rate of 1 mL/min with a gradient from 0 % to 70 % of B in 30 min (eluent A: $H_2O$/0.1% TFA; eluent B: $CH_3CN$/0.1% TFA) for the crudes, and a gradient from 5 % to 95 % of B in 15 min for the analysis of the pure peptides. Purification of the final peptides was performed using a semipreparative *Agilent Zorbax 300 SB-C3 column* (5 µm, 300 Å, 250 × 9.4 mm) at a flow rate of 4 mL/min employing the following gradient: 0 to 5 min, 0% B; 5 to 85 min, 0 to 70% B. Eluent A: $H_2O$/0.1% TFA; eluent B: $CH_3CN$/0.1% TFA.

These standard conditions were used otherwise indicated. Compounds were monitored at 222, 270, 304 and 330 nm (and 555 nm for peptides containing TAMRA moieties)

### 4.2. Electrospray Ionization Mass Spectrometry (ESI/MS)

ESI-MS was performed with an *Agilent 1100 Series* LC/MSD VL G1956A model in positive scan mode, using direct injection of the purified peptide solution into the mass spectrometer.

### 4.3. NMR

NMR spectra were recorded using a *Varian Mercury 300* (300 MHz for $^{1}H$ and 75 MHz for $^{13}C$) spectrophotometer and processed using the *MestReNova* v6.1.1-6384 suite (Mestrelab Research).

### 4.4. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI/MS)

MALDI spectrometry was performed with a Bruker Autoflex MALDI/TOF model in positive scan mode by direct irradiation of the matrix- absorbed peptide

### 4.5. Circular Dichroisms (CD)

Circular dichroism measurements were performed on a *Jasco J-715* equipment coupled to a *Neslab RTE-111* thermostatic water bath, using a *Hellma* micro cuvette (100-QS, 2 mm light pass). Spectra were slightly smoothed for a better visualization using the macro "*smooth*", implemented in the progr am KaleidaGraph (Synergy Software version 4.1).

Measurements of a 5 µM solution of peptide (300 µL) were run in 10 mM phosphate, 150 mM KCl, 1mM DTT, and 0.25 mM EDTA buffer pH 7.4, at 20 º C. The spectra are the average of 4 scans

Settings:

- Acquisition range: 380 – 195 nm.
- Band width: 2.0 nm.
- Resolution: 0.2 nm.
- Sensitivity: 10 milidegrees.
- Response time: 0.25 seconds.
- Speed: 100 nm/ min.

### 4.6. Quantification of proteins and oligonucleotides using UV

UV measurements were performed on a *Varian Cary 100 Bio spectrophotometer* or a *JASCO UV-630* spectrophotometer coupled with a *PolyScience* thermostat, using a standard *Hellma* semi-micro cuvette (140.002-QS). The concentrations of the stock solutions of the peptides or DNA were obtained using the Lambert Beer's law.

For peptides **[AQ]MYC(SH)**, **[AQ]MYC**, **[AQ]MYC(S-S),** MAX and $^{wt}$**MYC**, Tyr was used as the chromophore with a molar extinction coefficient of 1405 $M^{-1}$ $cm^{-1}$ at 274 nm

For labelled peptides *TMR*-**[AQ]MYC,** *TMR*-**[AQ]MYC(SH),** *TMR*-**[AQ]OMOMYC** and *TMR*-**[AQ]OMOMYC(SH),** TAMRA was used as the chromophore with a molar extinction coefficient of 80000 $M^{-1}$ $cm^{-1}$ at 552 nm.

DNA solutions were measured at 260 nm and the molar extinction coefficients (in $M^{-1}$ $cm^{-1}$) of single strand oligonucleotides were calculated with the following formula:[1]

$$\varepsilon_{260nm} = \{(8.8 \times T) + (7.3 \times C) + (11.7 \times G)+(15.4 \times A)\} \times 0.8\ 10^3$$

Where T, C, G, and A represent the number of the corresponding nitrogenated bases in the sequence of the DNA.

[1] a) K. C. Engman, P. Sandin, S. Osborne, T. Brown, M. Billeter, P. Lincoln, B. Norden, B. Albinsson and L. M. Wilhelmsson, *Nucl. Acids Res*. 2004, **17**, 5087-5095. b) G. Kallansrud and B. Ward, *Anal Biochem*. 1996, **236**, 134-138.

## 5. BACTERIAL TRANSFORMATION, SUBCLONATION, PURIFICATION OF GST PROTEINS AND CLEAVAGE OF GST PORTION.

Plasmids pBluescript p21 Max, pGEX Myc c92 and pGEX-2T-Myc C176 and the commercial one, pGEX 3X; were gently provided by *Bernhard Luscher (Germany)*.

Plasmids were amplified by bacterial transformation (using competent bacteria BL21). Cultures were spread on LB plates containing ampicillin and grown overnight. Colonies were picked for each DNA plasmid and placed in LB medium: after 16 h incubation at 37 ºC, DNA was purified using a midi prep kit acquired from *Promega* (PureYield plasmid Midiprep System).

To obtain the plasmid pGEX 3X p21 Max, the coding sequence of p21 Max was subcloned from pBluescript p21 Max into the pGEX-3X vector between the restriction sites for BamHI and EcoRI using standard cloning techniques. For this purpose, both plasmids were subjected to digestion using the restriction enzymes BamHI and EcoRI. After digestion and purification by agarose gel, DNA was extracted using the kit "Wizard SV gel and PCR Clean-up System", acquired from Promega. Then, ligation solutions containing the vector, the insert and the corresponding ligase (T4) and ligase buffer were prepared and after 16 h at 4ºC, ligation was transformed in competent bacteria BL21 and spread on LB plate containing ampicillin, grown in LB medium and finally, the construct was purified using a mini prep kit acquired also from *Promega* (Wizard Plus Minipreps).

For the purification of the GST-fusion proteins, the expression vectors were transformed into BL21 bacteria. One colony of transformed bacteria was used to inoculate a starter culture of 50 mL LB medium supplemented with 0.1 % (v/v) of ampiciline which was grown at 37ºC for 16h. The main culture was started by dilution of the starter culture at 1:15 in the same type of medium. The culture was grown at 37 ºC until an OD600 ≈ 0.5-0.7 was reached. Afterwards, expression of the GST fusion protein was induced by addition of 0.1 % (v/v) of 1 M IPTG. Expression was allowed for 2–3 h at 37 ºC. Subsequently, bacteria were pelleted by centrifugation at 3500 xg, resuspended in ice-cold TNE buffer (20 mM Tris·HCl pH 8.0. 150 mM NaCl, 1 mM EDTA, 5 mM DTT, 1 mM Pefabloc SC (Roche), 14 µg/ml aprotinin) and lyssed for 30 min on ice. Then the lysate was sonicated on ice and finally was cleared form cell debris by centrifugation for 10 min at 16000 xg (4 ºC). Then, proteins of interest were isolated from the lysates by affinity chromatography using Glutathione Sepharose 4B beads (GE Healtcare) –resin designed for the purification of glutathione S-transferase (GST) fusion proteins produced using the pGEX series of expression vectors, thanks to the affinity of glutathione for the GST tag following the manufacturer's instructions.

Before elution, fusion proteins GST-p21Max and GST-c92Myc were cleaved to release the GST portion by digestion with Factor Xa (New England Biolabs) and Thrombin (GE Healthacare) rescpectively.

Finally, proteins of interest (MAX and c92Myc, called $^{wt}$MYC herein after) were purified by RP-HPLC.

MAX: (3.5 mg/L)

ESI-MS (m/z): [MH]+ calculated for $C_{732}H_{1183}N_{235}O_{258}S_3$ = 17501.0; found 1347.2 $[MH_{13}]^{13+}$, 1167.2 $[MH_{15}]^{15+}$, 1094.4 $[MH_{16}]^{16+}$, 1030.3 $[MH_{17}]^{17+}$, 973.0 $[MH_{18}]^{18+}$, 921.7 $[MH_{19}]^{19+}$, 875.8 $[MH_{20}]^{20+}$

MALDI: [MH]+ calculated for $C_{732}H_{1183}N_{235}O_{258}S_3$ = 17501.0, found 17502.0 $[MH]^+$, 8759.4 $[MH_2]^{2+}$

$R_t$ = 16.3 min (column Eclipse XDB-C18, lineal gradient 5 to 95% of $CH_3CN$, 0.1 % TFA / $H_2O$, 0.1 % TFA in 30 min).

UV ($H_20$) $\lambda_{max}$: 274 nm; ε: 5620 $M^{-1}cm^{-1}$.[2]

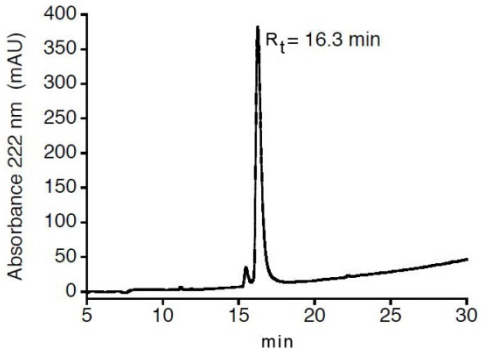



**Figure S1.** HPLC chromatogram of the pure protein at 222 nm**.**

$^{WT}$MYC**:** (7.2 mg/L).[3]

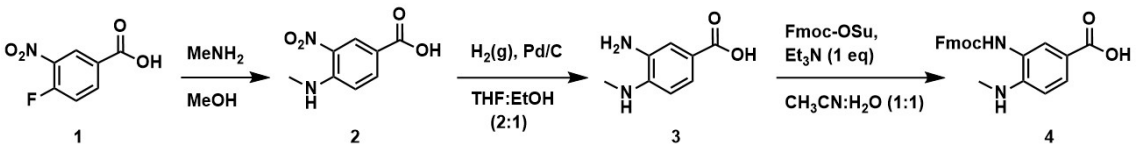
MALDI: [MH]+ calculated for $C_{488}H_{821}N_{155}O_{152}S$ = 11324.8, found 11320.8 $[MH]^+$, 5662 $[MH_2]^{2+}$

UV ($H_20$) $\lambda_{max}$: 274 nm; ε: 1405 $M^{-1}cm^{-1}$.

[2] The coefficient was calculated considering that the protein has four Tyr residues in the sequence.

[3] Besides our efforts, ESI-MS spectrum was not obtained for this protein.

## 6. SYNTHESIS OF 3-FMOC-4-DIAMINOBENZOIC ACID (4)




**Scheme 1.** Synthetic scheme for Fmoc-Dbz-OH.

Synthesis of intermediate **3** was performed as previously described.[4]

Final protection of the amino group was carried out using Fmoc-OSu: 1.7 g (10.5 mmol) of intermediate **3** and 3.9 g (11.57 mmol) of 9-Fluorenylmethyl *N*-succinimidyl carbonate (Fmoc-OSu) were dissolved in 100 mL (10.52 mmol) of $CH_3CN$: $H_2O$ (1:1). After $Et_3N$ addition (1.5 mL) the solution color changed to brown. The reaction was stirred for 24 h, and then acidified using 1 M HCl (aq) to get a precipitate that was filtered and washed with $CH_3CN$. The resulting solid was dried under vacuum and freeze-dried, affording the desired product as a white solid (3.2 g, 79 %).

$^1$H NMR (300 MHz, $d_6$- DMSO, 25ºC, TMS): δ=4.25-4.45 (m, 3H; CH, $CH_2$), 5.85 (s, 1H; NH), 6.60 (d, 1H; CH), 7.25-7.50 (m, 4H; CH), 7.65-7.80 (m, 4H; CH, 7.92 (d, 2H; CH), 8.70 (s, 1H; NH), 12.2 (s, 1H; OH).

ESI-MS (m/z): calculated for $C_{23}H_{20}N_2O_4$ $[M+1H]^+$: *m/z*=388.42, found 389.10

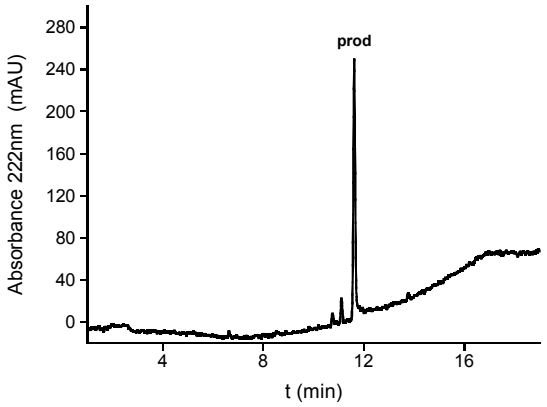



**Figure S2.** HPLC chromatogram of purified Fmoc-Dbz-OH at 222 nm.

[4] Kuzniweski, N. C; Gertsch, J.; Wartmann, M.; Altmann, H.K.; *Organic Letters* **2008**, *10*, 1183-1186

## 7. PROTOCOL FOR THE SYNTHESIS OF PEPTIDES

### 7.1. General protocol for solid phase peptide synthesis (SPPS)

Peptide fragments were synthesized by SPPS using an **automated microwave Liberty Blue Peptide Synthesizer** from *CEM Corporation* following the manufacturer recommended procedures. DIC (*N,N'*-Diisopropylcarbodiimide), was used as activator and Oxyma (Ethyl(hydroxyimino)cyanoacetate) as base. Peptides were synthesized at 0.1 mmol scale using the Fmoc strategy and *Rink Amide-ChemMatrix* (loading 0.5 mmol/g) as solid support. The deprotection of the temporal Fmoc protecting group was performed by treating the resin with 20 % piperidine in DMF. For sequences where aspartimide formation was a concern, 0.1 M HOBt was added to the deprotection cocktail to minimize the side reaction.

### 7.2. Cleavage/ deprotection step

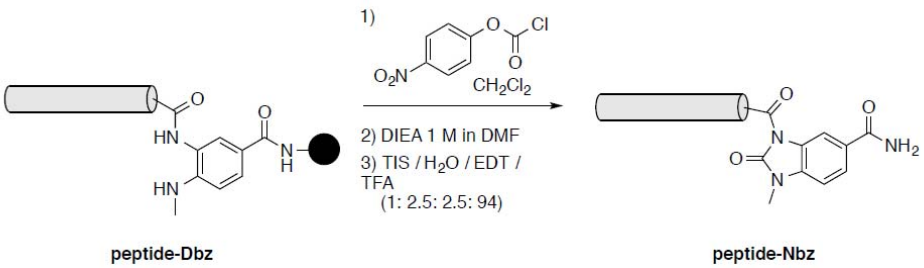
The final peptides were cleaved from the resin, and side-chain protecting groups were simultaneously removed using the following acidic cleavage cocktail: 94 % TFA, 2.5 % EDT, 2.5 % TIS and 1.0 % $H_2O$ (~1 mL/40 mg peptidyl-resin) for 3 h. The cleavage solution containing the peptide was concentrated under vacuum to a minimal volume, added over cold ether for precipitation and separated by centrifugation. The supernatant was removed and the residue was dissolved in 30% $CH_3CN$ in 0.1% aqueous TFA and freeze-dried. The resulting solid was purified by HPLC using a semipreparative column to obtain the desired peptide as a slid. The yield for the purified peptides was calculated based on the original resin loading and the amount of peptidyl-resin cleaved.

### 7.3. Synthesis of peptides with Nbz at C-terminus

The Dbz moiety was coupled manually to the resin as first *C*-terminal amino acid. For this, HATU (10 mmol) and Fmoc-Dbz-OH (1 mmol) were dissolved in DMF (0.2 M final concentration) and DIEA was added to the mixture (1.1 mmol). The amino acid was activated for 2 min before being added onto the resin. The resin was mixed by nitrogen bubbling during the couplings (60 min).

Then, the following amino acids of the fragment were coupled automatically using the general protocol for solid phase peptide synthesis described above. The last residue of this fragment was introduced as Boc-aa.

The transformation of Dbz in Nbz was carried out following previously described conditions:[5]




[5] Blanco-Canosa, B.J; Dawson, E.P.; *Angew. Chem. Int. Ed.* **2008**, *47*, 6851-6855

The *C*-terminus of the Dbz peptides was activated through acylation with 4-nitrophenylchloroformate followed by the addition of base to promote intramolecular attack of the anilide and form the resin-bound benzimidazolinone. For this purpose, ~0.1 mmol of each peptide resin was swelled in $CH_2Cl_2$ for 2 h. Then, the resin was filtered and a solution of 4-nitrophenylchloroformate (201 mg, 1 mmol) in $CH_2Cl_2$ (2.5 mL) was added over it. The mixture was shaken for 2 h and the resin was filtered and washed with $CH_2Cl_2$ (2 × 5 mL × 3 min) and DMF (2 × 5 mL × 3 min). The resin was then swelled in DMF for 25 min and, after filtration, a solution of DIEA 1 M in DMF was added onto it. $N_2$ (g) was passed through the resin suspension for 2 h. The resin was filtered and washed with DMF (2 × 5 mL × 3 min) and $CH_2Cl_2$ (2 × 5 mL × 3 min).

The final Nbz-peptides were cleaved from the resin, and side-chain protecting groups were simultaneously removed using the previously mentioned TFA cleavage cocktail (TFA: EDT: TIS: $H_2O$, 1 mL/40 mg peptidyl-resin) for 3 h. The TFA solution containing the peptide was concentrated under vacuum to a minimal volume, added over cold ether and precipitated by centrifugation. The supernatant was removed and the residue was dissolved in $CH_3CN$:$H_2O$ (3:7) and freeze-dried. The desired products were obtained after purification.

### 7.4. Introduction of TAMRA at the N-terminus

After SPPS synthesis, an extra Fmoc-Ava-Gly-Gly chain was added as a linker to the last *N*-terminal deprotected amino group of the corresponding fragment to avoid any side problem in the basic region of the peptides.

Then, TAMRA-OSu (1.5 eq) was dissolved in DMF (20 mM). HATU (1.5 eq) and *N, N*-diisopropylethylamine (4 eq DIEA) were added to this solution. After two minutes, the resulting mixture was added over the Fmoc-deprotected peptide attached to the resin (approximately 0.025 mmol, 1 eq). $N_2$ was passed through the resin suspension for 4 h at rt. After that, the resin was filtered, washed with DMF (3 × 3 mL × 3 min) and with $CH_2Cl_2$ (2 × 5 mL × 3 min).

The reaction product was checked after cleavage of a small resin aliquot (4 mg) by treatment with 100 µL of the cleavage reagent: TIS (25 µL), $H_2O$ (25 µL), $CH_2Cl_2$ (50 µL) and TFA (900 µL) for 2 hours, and its subsequent HPLC-MS analysis.

## 8. CHARACTERIZATION OF SYNTHETIC PEPTIDES

### $C^{537}L^{581}$ : CPKVVILKKATAYILSVQAEEQKLISEEDLLRKRREQLKHKLEQL-$NH_2$

This product was obtained using the *general protocol for solid phase peptide synthesis* previously described. After cleavage from resin and purification by RP-HPLC, the peptide was isolated as a white powder (27 %).

ESI-MS (m/z): $[MH]^+$ calculated for $C_{237}H_{411}N_{68}O_{67}S$= 5316.36; found 1773.0 $[M+3H]^{3+}$, 1329.9 $[M+4H]^{4+}$, 1064.2 $[M+5H]^{5+}$, 887.1 $[M+6H]^{6+}$, 760.4 $[M+7H]^{7+}$, 665.5 $[M+8H]^{8+}$.

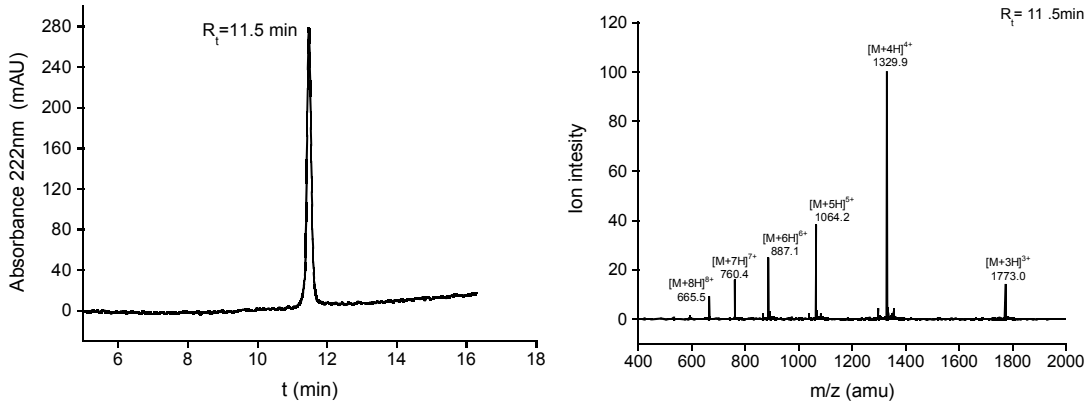



**Figure S3. Left:** HPLC chromatogram at 222 nm of the pure peptide $C^{537}L^{581}$. **Right:** ESI mass spectrum of the product.

### $C^{548}Leu^{581}$ : CYILSVQAEEQKLISEEDLLRKRREQLKHKLEQL-$NH_2$

This fragment was obtained following the *general protocol for solid phase peptide synthesis* previously described. The cleavage of 0.02 mmol of resin afforded the product $C^{548}L^{581}$ (37 mg, 45 %) as a white solid, that was used without further purification.

ESI-MS (m/z): $[MH]^+$ calculated for $C_{182}H_{310}N_{54}O_{55}S$= 4166.86, found 1464.2 $[M+3H]^{3+}$, 1098.2 $[M+4H]^{4+}$, 878.3 $[M+5H]^{5+}$

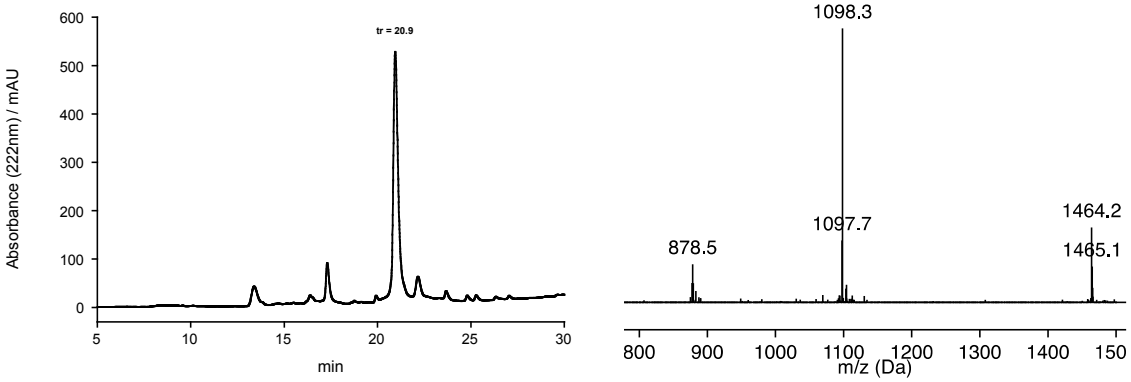



**Figure S4. Left:** HPLC chromatogram of the crude peptide $C^{548}L^{581}$ after cleavage from the resin at 222 nm. **Right:** ESI mass spectrum of the product.

**$A^{500}K^{536}$:** AVKRRTHNVLERQRRQELKRSFFALRDQIPELENNEK-Nbz

ESI-MS (m/z): $[MH]^+$ calculated for $C_{207}H_{339}N_{72}O_{59}$= 4779.44; found 1593.8 $[M+3H]^{3+}$, 1195.8 $[M+4H]^{4+}$, 956.7 $[M+5H]^{5+}$, 797.4 $[M+6H]^{6+}$, 683.8 $[M+7H]^{7+}$, 598.5 $[M+8H]^{8+}$

This fragment was obtained following the general protocol for *synthesis of peptides with Nbz at C-terminus* previously described.

Before the transformation into Nbz-peptide, a sample of the peptide with the Dbz moiety at the C-terminus was cleaved from the resin and analyzed. The crude of the cleavage and MS containing the fragment **$A^{500}K^{536}$**-Dbz is shown in the next figure. Finally, after the transformation into Nbz, cleavage from resin and purification by RP-HPLC, **$A^{500}K^{536}$** was obtained as a white solid (28 %)

**$A^{500}K^{536}$**–Dbz**: AVKRRTHNVLERQRRQELKRSFFALRDQIPELENNEK**-Dbz

ESI-MS (m/z): $[MH]^+$ calculated for $C_{206}H_{339}N_{72}O_{58}$= 4752.42; found 1585.8 $[M+3H]^{3+}$, 1189.3 $[M+4H]^{4+}$, 951.5 $[M+5H]^{5+}$, 792.9 $[M+6H]^{6+}$, 475.3 $[M+10H]^{10+}$.

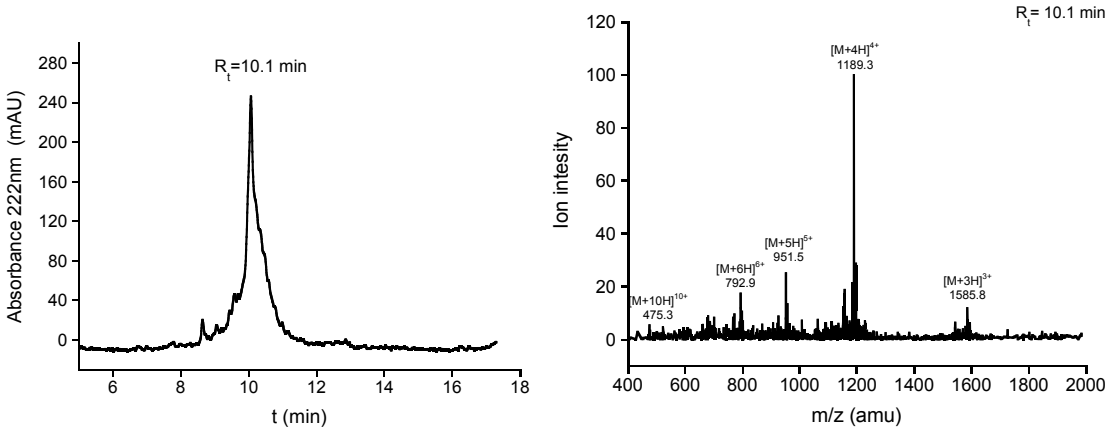



**Figure S5. Left:** HPLC chromatogram at 222 nm of the crude peptide with Dbz at C-terminus after cleavage from the resin. **Right:** ESI mass spectrum of the product.

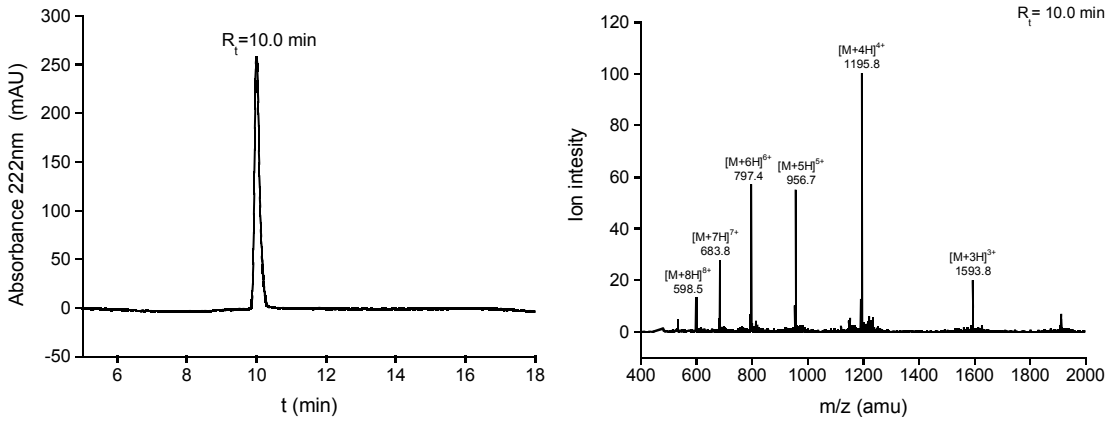



**Figure S6. Left:** HPLC chromatogram at 222 nm of the pure peptide with Nbz after purification. **Right:** ESI mass spectrum of the product.

**Thz$^{523}$T$^{547}$:** Thz-**PLRDQIPELENNEKAPKVVILKKAT**-Nbz

This fragment was obtained following the previously described *general protocol for synthesis of peptides with Nbz at C-terminus*. After cleavage of 0.05 mmol from resin the resulting white solid was used without further purification (45 mg, 29 %).

ESI-MS (m/z): [MH]+ calculated for $C_{135}H_{224}N_{38}O_{39}S$= 3035.56, found 1012. $[M+3H]^{3+}$, 783.0 $[M+4H]^{4+}$, 626.5 $[M+5H]^{5+}$

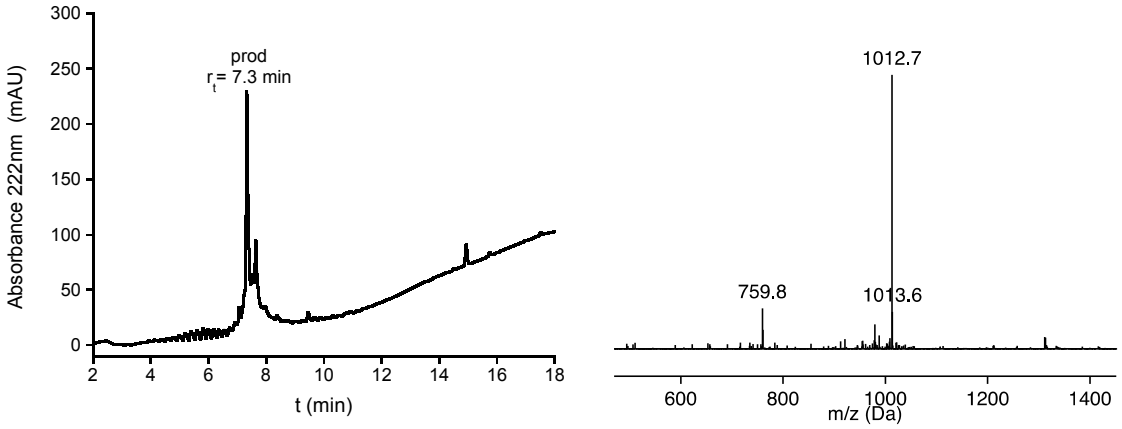



**Figure S7. Left:** HPLC chromatogram at 222 nm of the crude peptide **Thz$^{523}$T$^{547}$** after cleavage from the resin. **Right:** ESI mass spectrum of the product.

**C$^{548}$A$^{586}$ : CYILSVQAETQKLISEIDLLRKQNEQLKHKLEQLRNSSA**-$NH_2$

This fragment, needed for the preparation of OMOMYC peptides, was obtained following the *general protocol for solid phase peptide synthesis* previously described. The cleavage of 0.06 mmol from resin afforded the product **C$^{548}$A$^{586}$** (33 mg, 12 %) as a white solid that was used without further purification.

ESI-MS (m/z): $[MH]^+$ calculated for $C_{198}H_{337}N_{59}O_{62}S$= 4568.28; found 1523.8 $[M+3H]^{3+}$, 1142.8 $[M+4H]^{4+}$, 914.5 $[M+5H]^{5+}$.

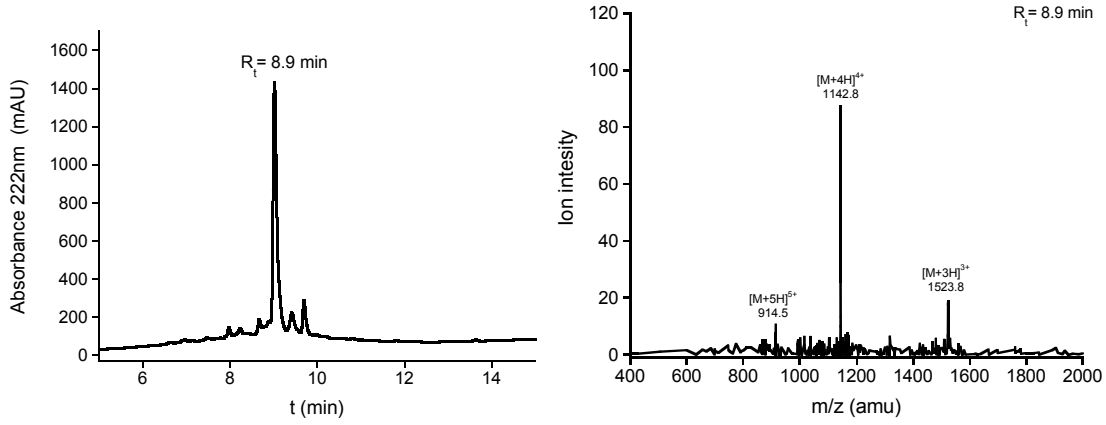



**Figure S8. Left:** HPLC chromatogram of the crude peptide **C$^{548}$A$^{586}$** after cleavage from the resin at 222 nm. **Right:** ESI mass spectrum of the product.

**TMR-AvaGG-A$^{500}$F$^{522}$–Nbz :** ***TMR*-AvaGG**AKRRTHNVLERQRRQELKRSFF-Nbz

This fragment, needed for the preparation of TMR-peptides, was obtained according to the previously described *general protocol for synthesis of peptides with Nbz at C-terminus* following the procedure of *introducing of TAMRA at the N-terminus*

ESI-MS (m/z): [MH]+ calculated for $C_{171}H_{258}N_{56}O_{401}$= 3754.30, found 1252.3 $[M+3H]^{3+}$, 939.5 $[M+4H]^{4+}$, 751.8 $[M+5H]^{5+}$, 626.8 $[M+6H]^{6+}$, 537.2 $[M+7H]^{7+}$

Before the transformation into the Nbz-peptide, a sample with the Dbz moiety was cleaved from the resin and analyzed. The crude of the cleavage and MS containing the peptide **AvaGG-A$^{500}$F$^{522}$**-Dbz is shown in the next figure. After TMR coupling and Nbz formation, the resulting peptide was cleavage from the resin. Purification by RP-HPLC afforded *TMR*-**AvaGG-A$^{500}$F$^{522}$**-Nbz as a pink solid (16 %)

**AvaGG-A$^{500}$F$^{522}$–Dbz : AvaGG**AKRRTHNVLERQRRQELKRSFF-Dbz

ESI-MS (m/z): [MH]+ calculated for $C_{145}H_{240}N_{54}O_{36}$= 3315.86, found 1106.3 $[M+3H]^{3+}$, 830.0 $[M+4H]^{4+}$, 664.2 $[M+5H]^{5+}$, 553.6 $[M+6H]^{6+}$

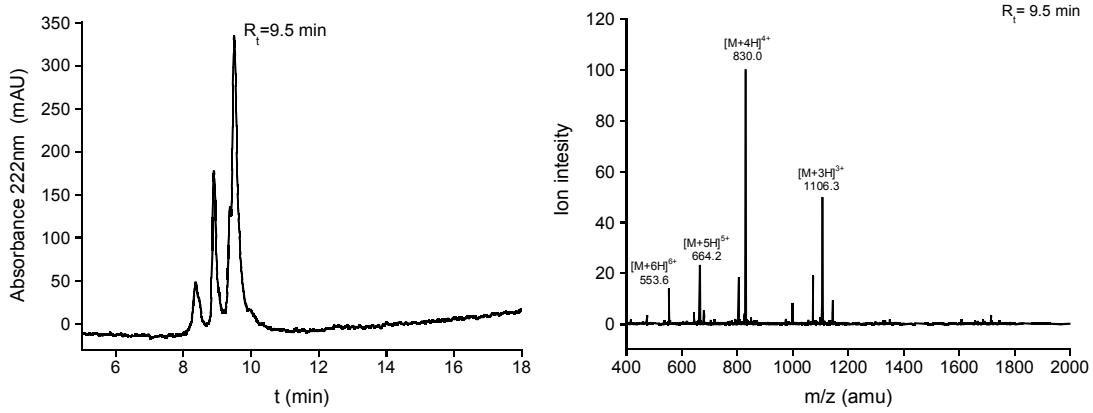



**Figure S9. Left:** HPLC chromatogram of the crude peptide **AvaGG-A$^{500}$F$^{522}$**-Dbz after cleavage from the resin at 222 nm. **Right:** ESI mass spectrum of the product.

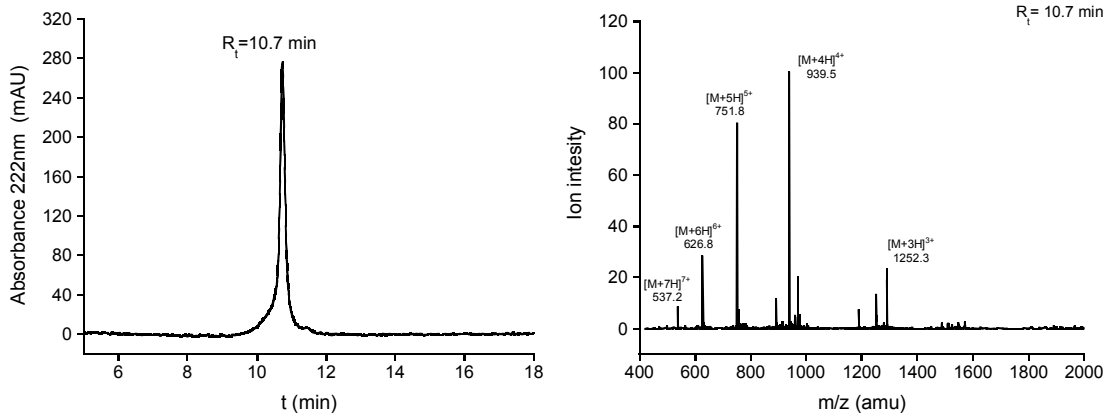



**Figure S10. Left:** HPLC chromatogram of the pure peptide **TAMRA-AvaGG-A$^{500}$F$^{522}$**-Nbz after cleavage from the resin and purification by RP-HPLC, at 222 nm. **Right:** ESI mass spectrum of the product.

## 9. GENERAL PROTOCOL FOR NATIVE CHEMICAL LIGATIONS

The *Native Chemical Ligations* were performed at room temperature by dissolving the Nbz-peptide (2.5 mM) and adding 1.5 equiv of the cysteinyl peptide in 6 M Gn·HCl, 200 mM phosphate buffer (pH 7.0). 4-mercaptophenylacetic acid (MPAA) and tris(2-carboxyethyl) phosphine hydrochloride (TCEP·HCl) were then added to final concentrations of 100 mM and 40 mM, respectively. The pH was then adjusted to 7.5 with NaOH (aq) and the buffer solution was degassed under $N_2$ (g) for 15 min.

The progress of the reaction was monitored by HPLC-MS. Once the chromatographic analysis showed that the reaction was complete, it was quenched by diluting the crude up to 3 times with buffer, and freezing using liquid nitrogen.

Finally, the product was purified by HPLC. When needed, the crude was filtrated using *Amicon Ultra-0.5 mL centrifugal filters* for protein purification. Spin conditions for filtration: 40 degree fixed angle rotor, 14,000 g, room temperature, 500 µL starting volume, 20-15 µL final volume, 10-minute spin Spin conditions for sample recovery: reverse spinning, 30 µL starting volume, 40 degree fixed angle rotor, 1,000 g, room temperature, 4 min spin.

### 9.1. Thiazolidine deprotection protocol

When the orthogonal deprotection of the *N*-terminal Cys of the product resulting from the 1st NCL was needed, it was performed in one pot, right after the ligation reaction. After the NCL was completed, hydroxylamine was added (200 mM). The pH of the solution was then adjusted to 4. The solution was shaken for 14 h and the product was purified by RP-HPLC. The appropriate fractions were collected and freeze-dried.

### 9.2. Two-fragment approach. Synthesis of [AQ,C$^{537}$]MYC

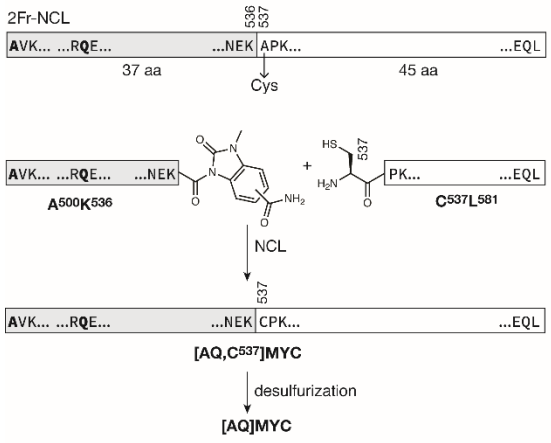



**Figure S11.** Two-fragment disconnection for synthesizing the Asn$^{500}$→Ala and Asn$^{515}$→Gln MYC mutant.

**[AQ, C$^{537}$]MYC**

**AVKRRTHNVLERQRRQELKRSFFALRDQIPELENNEKCPKVVILKKATAYILSVQAEEQK LISEEDLLRKRREQLKHKLEQL**-$NH_2$

Synthesized from **A$^{500}$K$^{536}$**-Nbz (15 mg, 3.1 µmol) and **C$^{537}$L$^{581}$** (25 mg, 4.7 µmol) following the general procedure for NCL. A. white powder was obtained after RP-HPLC purification (16 mg, 54 %)

ESI-MS (m/z): $[MH]^+$ calculated for $C_{435}H_{739}N_{137}O_{124}S$= 9904.59; found 1981.9 $[M+5H]^{5+}$, 1651.8 $[M+6H]^{6+}$, 1238.9 $[M+8H]^{8+}$, 1101.6 $[M+9H]^{9+}$, 991.3 $[M+10H]^{10+}$, 901.3 $[M+11H]^{11+}$ , 826.3 $[M+12H]^{12+}$, 762.8 $[M+13H]^{13+}$.

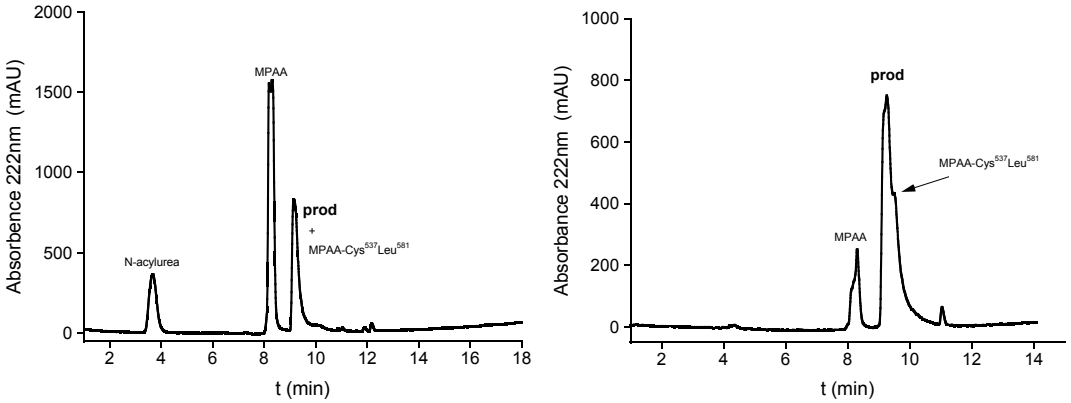



**Figure S12. Left:** HPLC at 222 nm of the crude reaction after 4.5 h. **Right:** HPLC of the crude reaction after filtered through amicon filters. **prod** refers to the peptide [**AQ,C$^{537}$] MYC**.

The *N*-terminal cysteine of the fragment **C$^{537}$L$^{581}$** in excess formed a disulfide impurity with the thiol MPAA. As the desired product and this adduct had the same retention time, TCEP was added to the mixture prior to the purification. The HPLC gradient was also changed for semipreparative purifications. The best chromatographic resolution was obtained using a gradient from 25 % to 45 % of eluent B during 30 min.

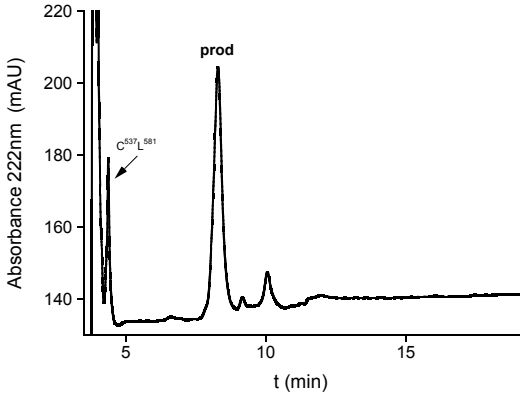



**Figure S13.** HPLC of the purification in the semipreparative HPLC after TCEP added. Gradient used: 25 % → 45 % of eluent B in 30 min. Prod refers to **[AQ,C$^{537}$]MYC** peptide

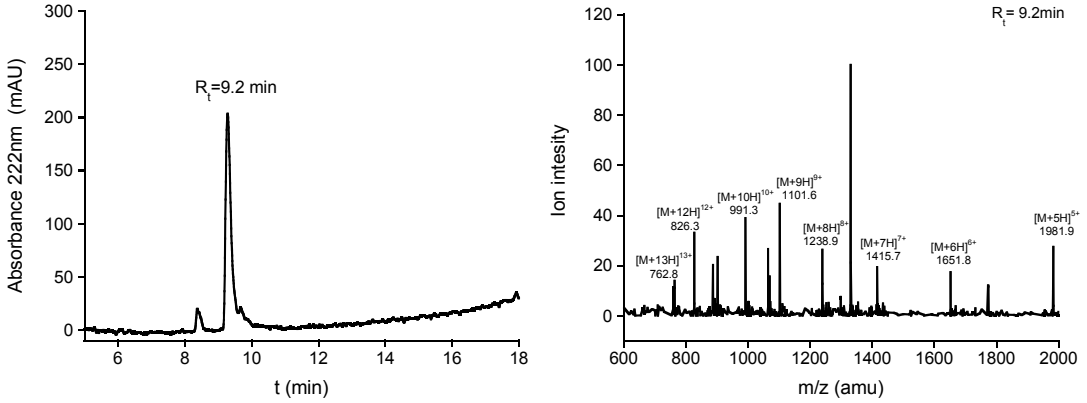



**Figure S14.** Left: HPLC of the pure **[AQ,C$^{537}$]MYC** peptide at 222 nm. **Right:** ESI mass spectrum of the product.

## 9.3. Three-fragment approach. Synthesis of [AQ,C$^{523}$,C$^{548}$]MYC= [AQ]MYC(SH)

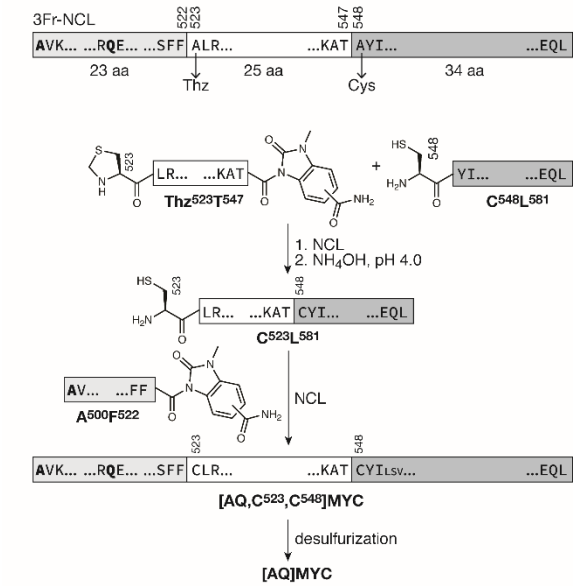



**Figure S15.** Three-fragment disconnections used for synthesizing the Asn$^{500}$→Ala and Asn$^{515}$→Gln MYC mutant

1$^{ST}$ NCL

**C$^{523}$L$^{581}$**

**CLRDQIPELENNEKAPKVVILKKATCYILSVQAEEQKLISEEDLLRKRREQLKHKLEQL**-$NH_2$

This NCL between fragments **Thz$^{523}$T$^{546}$**–Nbz (20 mg, 6.6 μmol) and **C$^{549}$L$^{581}$** (41 mg, 9.9 μmol) was carried out according to the general procedure for NCL. A. white powder (9 mg, 20 %) was obtained after thiazolidine removal and RP-HPLC purification.

ESI-MS (m/z): $[MH]^+$ calculated for $C_{307}H_{525}N_{89}O_{92}S_2$= 6999.23; found 1750.9 $[M+4H]^{4+}$, 1400.8 $[M+5H]^{5+}$, 1000.8 $[M+7H]^{7+}$, 1167.4 $[M+6H]^{6+}$, 875.8 $[M+8H]^{8+}$, 778.3 $[M+9H]^{9+}$.

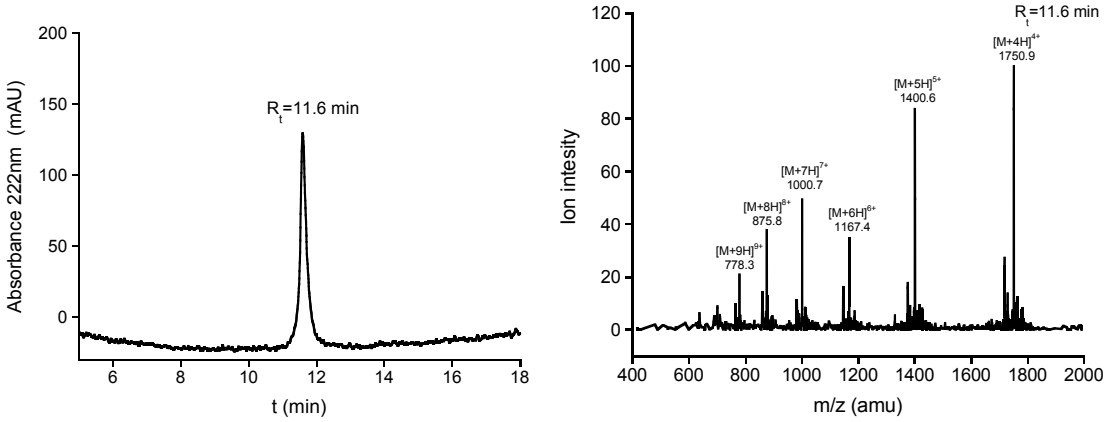



**Figure S16. Left:** HPLC of the pure peptide at 222 nm. **Right:** ESI mass spectrum of product.

*2nd NCL*

**[AQ]MYC(SH)**

**AVKRRTHNVLERQRRQELKRSFFCLRDQIPELENNEKAPKVVILKKATCYILSVQAEEQK LISEEDLLRKRREQLKHKLEQL**-$NH_2$

The 2nd NCL was performed between fragments **$C^{523}L^{581}$** (19 mg, 2.8 µmol) and **$A^{500}F^{522}$** (13 mg, 4.2 µmol). A white powder (7.5 mg, 27 %) was recovered after RP-HPLC purification

ESI-MS (m/z): $[MH]^+$ calculated for $C_{435}H_{739}N_{137}O_{124}S_2$= 9936.7, found 1242.7 $[M+8H]^{8+}$, 1105.1 $[M+9H]^9$, 903.6 $[M+11H]^{11+}$, 828.8 $[M+12H]^{12+}$, 765.0 $[M+13H]^{13+}$

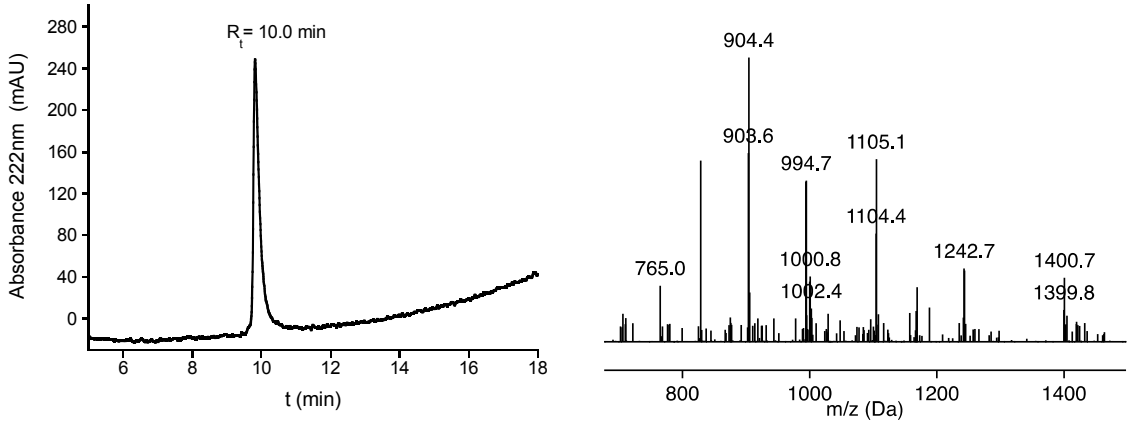



**Figure S17. Left:** HPLC of the pure peptide at 222 nm. **Right:** ESI mass spectrum of product.

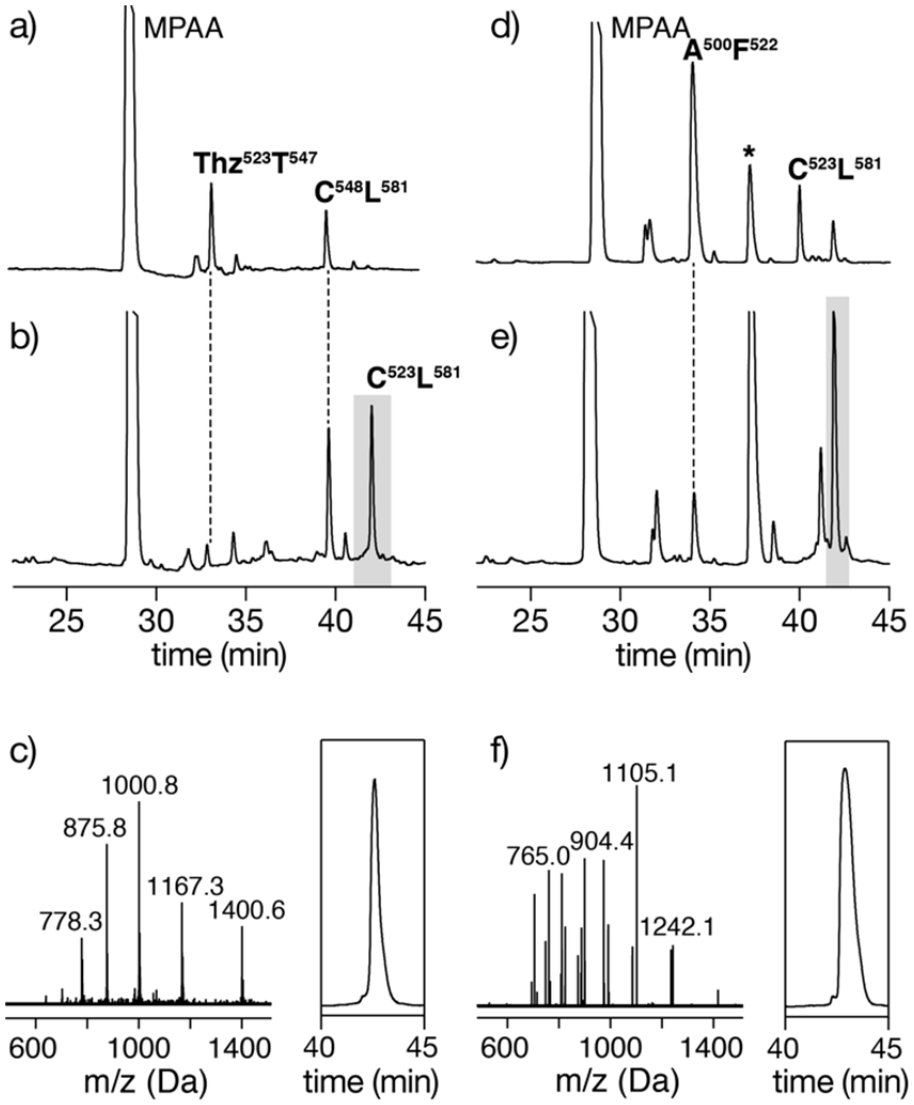



**Figure S18.** 3Fr-NCL strategy. *Left*: first NCL between peptide segments **C$^{548}$L$^{581}$** and **Thz$^{523}$T$^{547}$**. a) HPLC trace of the mixture at t = 5 min; b) same reaction after 4 h ligation, followed by Thz removal; c) **C$^{523}$L$^{581}$** after HPLC purification with the corresponding ESI-MS. *Right*: second NCL between *N*-terminal fragment **A$^{500}$F$^{522}$** and **C$^{523}$Q$^{581}$** (Figure 1). d) HPLC trace of the mixture at t = 5 min; e) same reaction after t= 2 h highlighting the desired product (**[AQ, C$^{523}$,C$^{548}$]MYC**); f) HPLC and MS of the purified product (see the SI† for details). The peak labeled with an asterisk corresponds to the thioester intermediate **A$^{500}$F$^{522}$**-MPAA.

## 9.4. Three fragment approach. Synthesis of *TMR*-[AQ]MYC

*2nd NCL*

*TMR*-**[AQ]MYC(SH)**

*TMR*-
**AvaGGAVKRRTHNVLERQRRQELKRSFFCLRDQIPELENNEKAPKVVILKKATCYILSVQ AEEQKLISEEDLLRKRREQLKHKLEQL**-$NH_2$

This ligation between fragments **[$C^{523}L^{581}$]** (14 mg, 2 µmol) and *TMR*-**AvaGG-$A^{500}F^{522}$**–Nbz (11 mg, 3 µmol) was carried out according to the general procedure for NCL. A pink powder (9.5 mg, 45 %) was obtained after RP-HPLC purification and lyophilization.

ESI-MS (m/z): $[MH]^+$ calculated for $C_{469}H_{774}N_{142}O_{131}S_2$= 10562.33; found 1761.39 $[M+6H]^{6+}$, 1509.8 $[M+7H]^{7+}$, 1321.0 $[M+8H]^{8+}$, 1174.4 $[M+9H]^{9+}$, 1056.9 $[M+10H]^{10+}$, 960.9 $[M+11H]^{11+}$, 881.0 $[M+12H]^{12+}$, 813.5 $[M+13H]^{13+}$

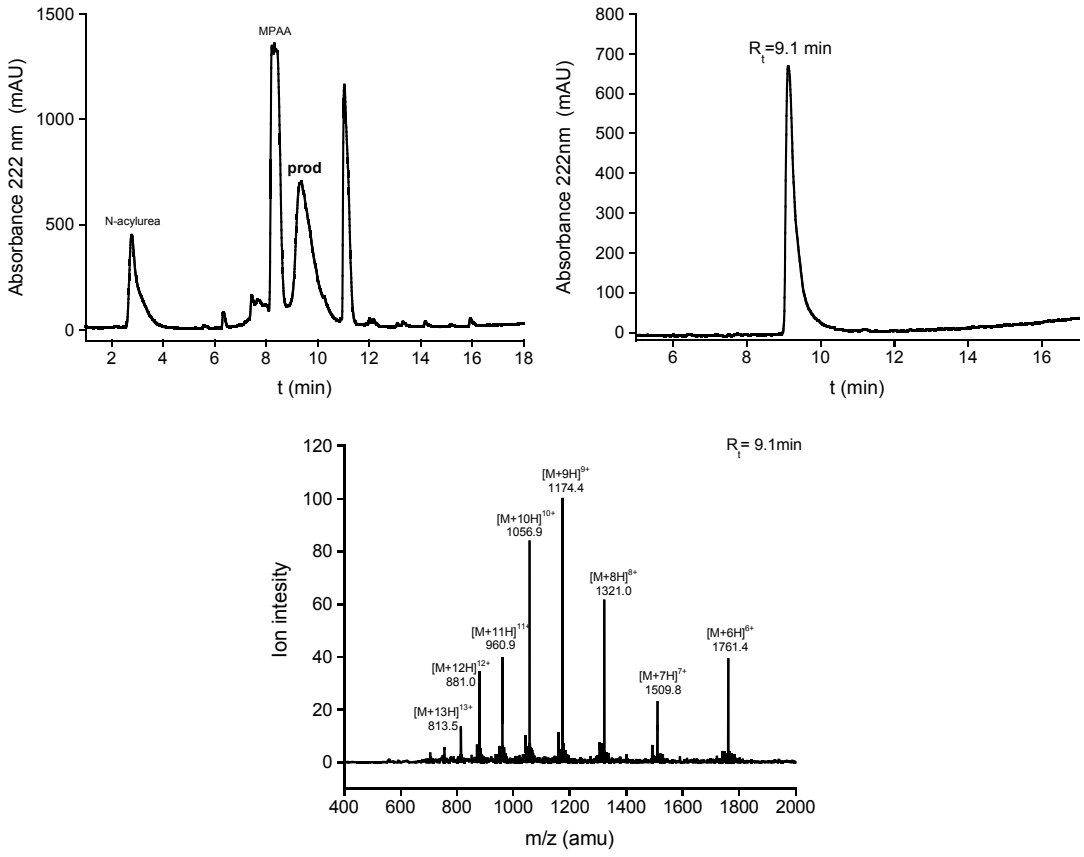
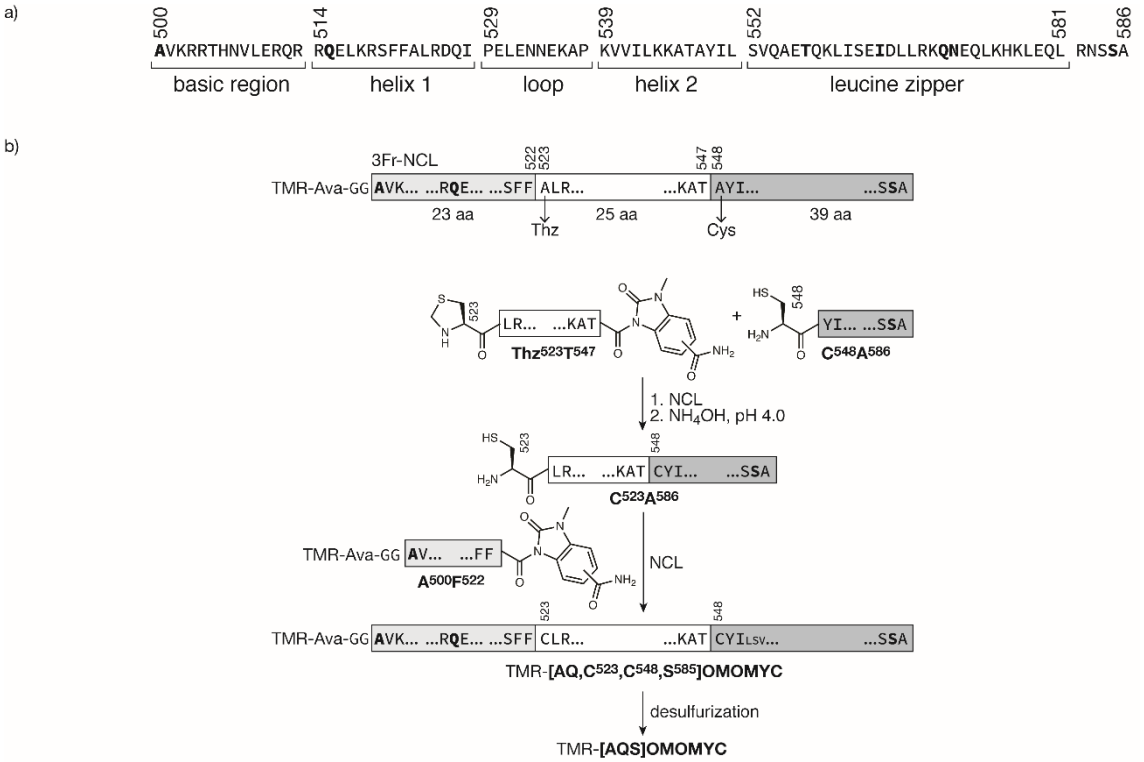



**Figure S19. Top left:** HPLC of the crude reaction after the 2nd NCL, at 222 nm; **Top right:** HPLC of the pure peptide at 222 nm. **Bottom:** ESI mass spectrum of product. **Prod** refers to the expected peptide *TMR*-[**AQ[MYC(SH)**

## 9.5. Three fragment approach. Synthesis of *TMR*-[AQS]OMOMYC(SH)




**Scheme 2.** a) Sequence and structural elements of the peptide *omomyc*; in bold indicated the amino acids that are different from MYC, as well as the two N-terminal mutations we had already introduced in our synthetic MYC. b) Three-fragment disconnection for synthesizing the $Asn^{500}$→Ala and $Asn^{515}$→Gln *omomyc* mutant (**[AQS]OMOMYC**). Numbering in the sequence maintains that of MYC for consistency.

*1ST NCL*

**$C^{523}A^{586}$**

**CLRDQIPELENNEKAPKVVILKKATCYILSVQAETQKLISEIDLLRKQNEQLKHKLEQLRN SSA**-$NH_2$

This ligation involved the fragments **$Thz^{523}T^{546}$**–Nbz (5 mg, 1.8 µmol) and **$C^{549}L^{581}$** (10 mg, 2.2 µmol). In this case, the best yield was achieved adding only 1.2 equivalents of the last reactant. After following the general procedure for NCL and thiazolidine deprotection, a white powder (6 mg, 43 %) was obtained.

ESI-MS (m/z): $[MH]^+$ calculated for $C_{323}H_{552}N_{94}O_{99}S_2$= 7400.65; found 1234.6 $[M+6H]^{6+}$, 1058.1 $[M+7H]^{7+}$, 925.9 $[M+8H]^{8+}$, 823.2 $[M+9H]^{9+}$, 741.1 $[M+10H]^{10+}$.

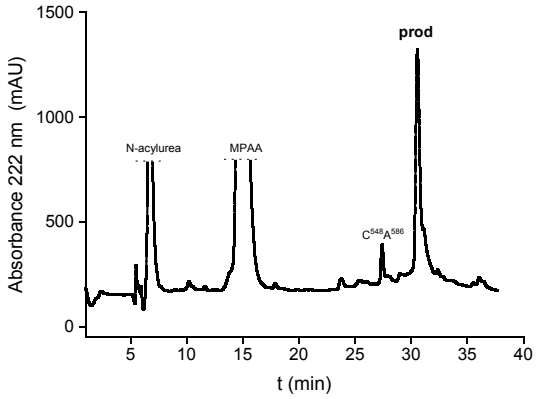



**Figure S20.** HPLC of the crude reaction after the 1st NCL and thiazolidine deprotection, at 222 nm. Gradient used: 20 % → 65 % in 70 min. **prod** refers to **$C^{523}A^{586}$**.

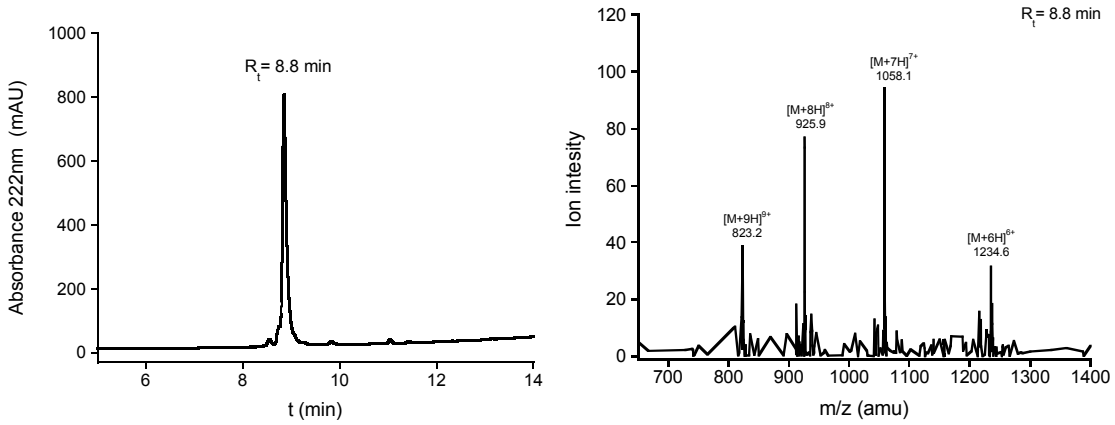



**Figure S21. Left:** Chromatogram at 222 nm of the pure peptide after the 1st NCL and thiazolidine deprotection. **Right:** ESI mass spectrum of pure product.

*2nd NCL*

***TMR*-[AQC$^{523}$C$^{548}$S$^{585}$] OMOMYC = *TMR*-[AQS]OMOMYC(SH)**

***TMR*-**
**AvaGGAVKRRTHNVLERQRRQELKRSFFCLRDQIPELENNEKAPKVVILKKATCYILSVQ**
**AETQKLISEIDLLRKQNEQLKHKLEQLRNSSA**-$NH_2$

This ligation took place between fragments **[C$^{523}$A$^{586}$]** (7 mg, 1 µmol) and ***TMR*-AvaGG-A$^{500}$F$^{522}$**–Nbz (6 mg, 1.5 µmol) according to the general procedure for NCL. The product was obtained as a pink powder (4.2 mg, 39 %) after RP-HPLC purification and lyophilization

ESI-MS (m/z): $[MH]^+$ calculated for $C_{485}H_{801}N_{147}O_{138}$= 10899.63; found 1371.8 $[M+8H]^{8+}$, 1219.2 $[M+9H]^{9+}$, 1097.4 $[M+10H]^{10+}$, 997.8 $[M+11H]^{11+}$, 914.5 $[M+12H]^{12+}$, 844.0 $[M+13H]^{13+}$, 784.3 $[M+14H]^{14+}$.

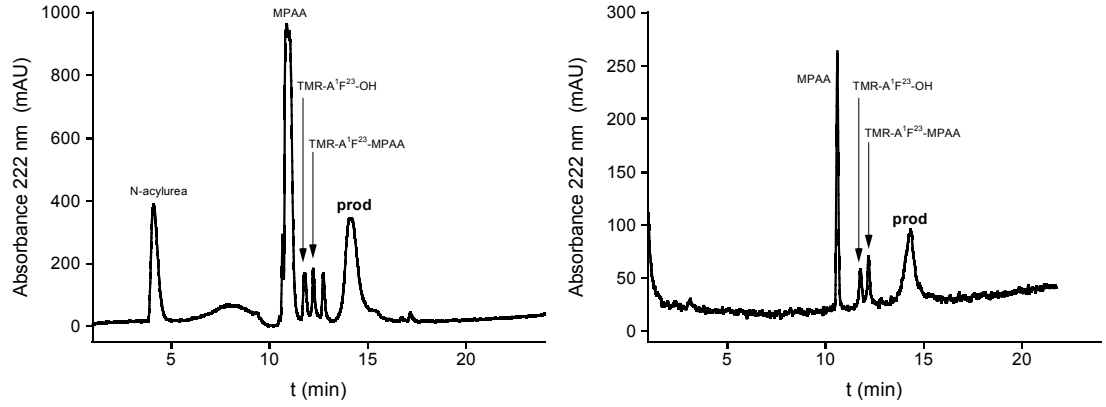



**Figure S22. Left**: HPLC at 222 nm of the crude reaction after the 2nd NCL. Gradient used: 5 % → 75 % of B in 19 min. **Right:** Crude of the reaction after amicon filtering. **Prod** refers to *TMR*-**[AQS]OMOMYC(SH)**.

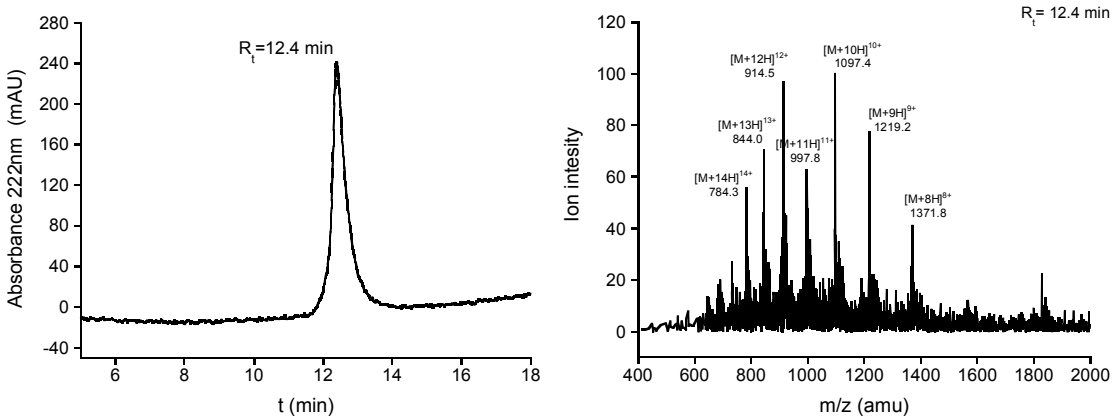



**Figure S23. Left:** HPLC at 222 nm of the pure product. Gradient used: 5 % → 95 % of B in 19 min. **Right:** MS spectrum of the product.

## 10. DESULFURIZATION OF THE CYSTEIN-CONTAINING SYNTHETIC MINIPROTEINS

0.3 µmol of the corresponding peptide was dissolved in degassed 6 M Gn·HCl, 200 mM $K_2HPO_4$ and 500 mM TCEP (pH = 7) to a 100 µM concentration. After degassing the resulting mixture for 10 min, 4.6 % (v/v) of EtSH and 40 mM of VA-044 (aq) were added. Reaction mixture was shaken at 37 ºC for 5.5 hours. Then, it was quenched with TFA and directly purified by RP-HPLC to afford the desired product.

### 10.1. Desulfurization of [AQ,C$^{537}$]MYC in the 2-fragment approach

**[AQ]MYC**

**AVKRRTHNVLERQRRQELKRSFFALRDQIPELENNEKAPKVVILKKATAYILSVQAEEQK LISEEDLLRKRREQLKHKLEQL**-$NH_2$

The product was obtained as a white solid (2.2 mg, 76 %) from pure starting peptide was recovered after purification.

ESI-MS (m/z): $[MH]^+$ calculated for $C_{435}H_{739}N_{137}O_{124}$= 9872.53; found 1975.6 $[M+5H]^{5+}$, 1646.1 $[M+6H]^{6+}$, 1411.2 $[M+7H]^{7+}$, 1234.9 $[M+8H]^{8+}$, 1097.6 $[M+9H]^{9+}$, 988.0 $[M+10H]^{10+}$, 898.7 $[M+11H]^{11+}$ , 823.8 $[M+12H]^{12+}$, 760.4 $[M+13H]^{13+}$

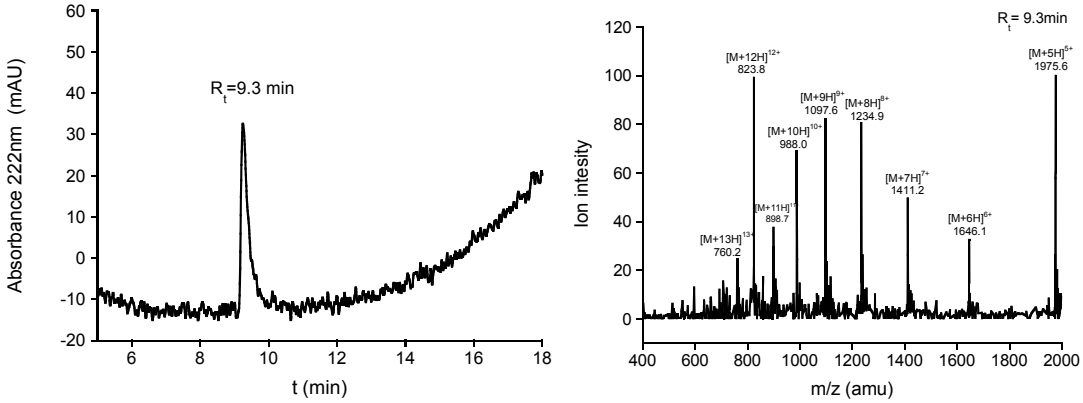



**Figure S24. Left:** HPLC of the pure peptide at 222 nm. **Right:** ESI mass spectrum of product.

## 10.2. Desulfurization of [AQ]MYC(SH) in the 3-fragment approach

**[AQ]MYC**

**AVKRRTHNVLERQRRQELKRSFFALRDQIPELENNEKAPKVVILKKATAYILSVQAEEQK LISEEDLLRKRREQLKHKLEQL**-$NH_2$

The desired product was obtained as a white solid (2.1 mg, 72 %).

ESI-MS (m/z): $[MH]^+$ calculated for $C_{435}H_{739}N_{137}O_{124}$= 9872.37, found 1234.5 $[M+8H]^{8+}$, 1097.5 $[M+9H]^{9+}$, 988.8 $[M+10H]^{10+}$, 898.2 $[M+11H]^{11+}$, 823.0 $[M+12H]^{12+}$,760.1 $[M+13H]^{13+}$, 658.4 $[M+14H]^{14+}$

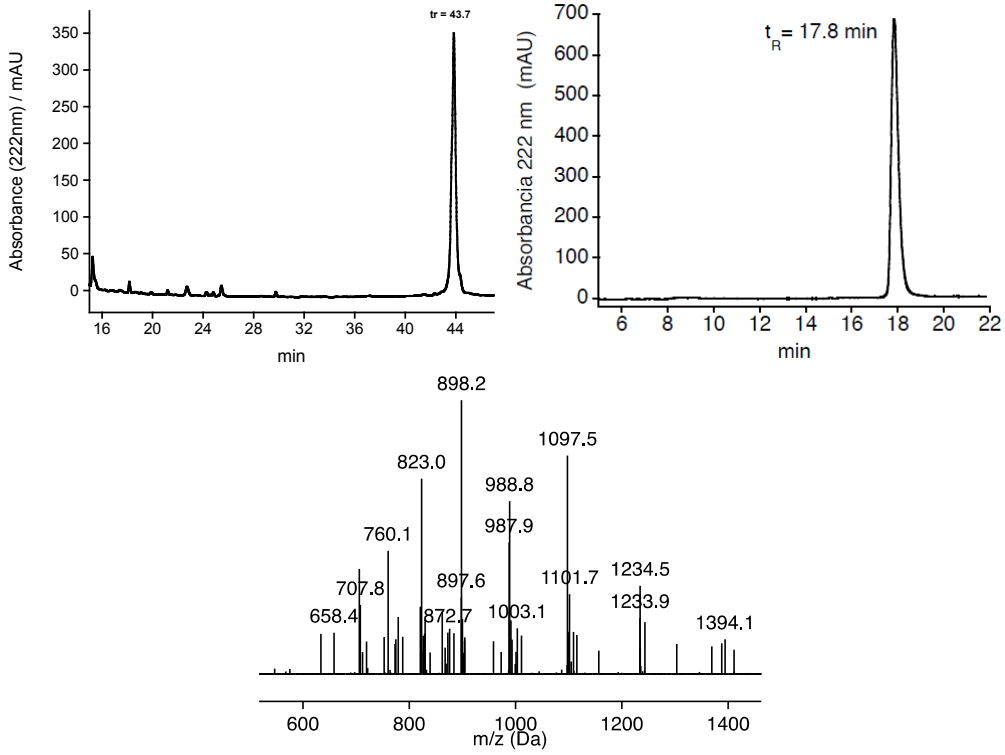



**Figure S25. Top left:** HPLC of the reaction crude after 5 h. **Top right:** HPLC of the pure product at 222 nm. **Bottom:** ESI mass spectrum of the reaction product.

### 10.3. Desulfurization of TMR-[AQ]MYC(SH) in the 3-fragment approach

*TMR*-**[AQ]MYC**

*TMR*-
**AvaGGAVKRRTHNVLERQRRQELKRSFFALRDQIPELENNEKAPKVVILKKATAYILSVQ AEEQKLISEEDLLRKRREQLKHKLEQL**-$NH_2$

After purification, the pure peptide (2.2 mg, 70 %) was isolated as a pink solid.

ESI-MS (m/z): $[MH]^+$ calculated for $C_{469}H_{774}N_{142}O_{131}$= 10498.23; found 1750.8 $[M+6H]^{6+}$, 1500.0 $[M+7H]^{7+}$, 1313.3 $[M+8H]^{8+}$, 1167.0 $[M+9H]^{9+}$, 1051.4 $[M+10H]^{10+}$, 954.2 $[M+11H]^{11+}$, 876.1 $[M+12H]^{12+}$, 751.9 $[M+14H]^{14+}$

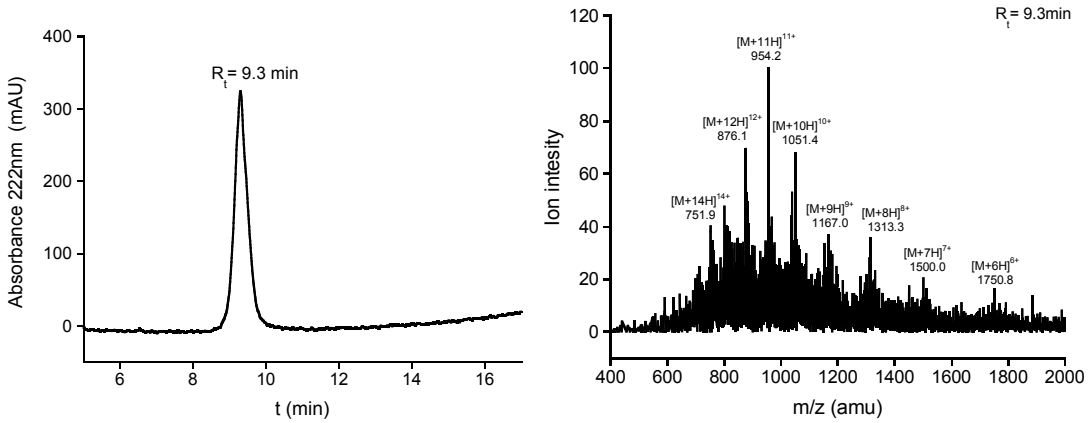



**Figure S26. Left:** HPLC of the pure peptide at 222 nm. **Right:** ESI mass spectrum of product.

### 10.4. Desulfurization of *TMR*-[AQS]OMOMYC(SH) obtained by the 3-fragment approach

*TMR*-**[AQS]OMOMYC**

*TMR*-
**AvaGGAVKRRTHNVLERQRRQELKRSFFALRDQIPELENNEKAPKVVILKKATAYILSVQ AETQKLISEIDLLRKQNEQLKHKLEQLRNSSA**-$NH_2$

After RP-HPLC purification, pure peptide was obtained as a pink solid (2.6 mg, 80 %).

ESI-MS (m/z): $[MH]^+$ calculated for $C_{485}H_{801}N_{147}O_{138}$= 10899.63; found 1817.5 $[M+6H]^{6+}$, 1558.0 $[M+7H]^{7+}$, 1212.0 $[M+9H]^{9+}$, 1091.0 $[M+10H]^{10+}$, 991.7 $[M+11H]^{11+}$, 909.4 $[M+12H]^{12+}$, 839.3 $[M+13H]^{13+}$, 779.5 $[M+14H]^{14+}$

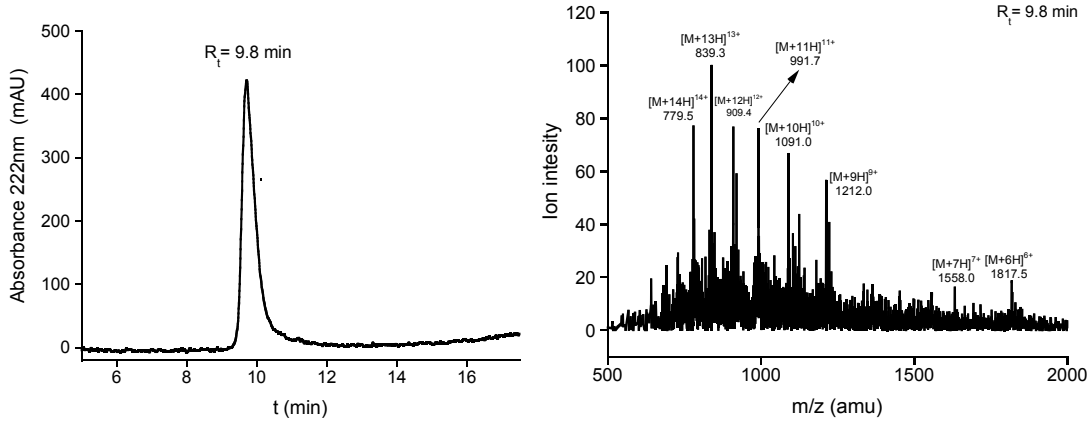



**Figure S27. Left:** HPLC chromatogram of the pure desulfurized peptide at 222 nm**. Right:** ESI mass spectrum of the peptide.

## 11. SYNTHESIS OF THE DISULFIDE DIMER [AQ]MYC(S-S)

The thiol containing peptide **[AQ]MYC(SH)** (2 mg, 0.2 μmol) was dissolved in 100 mM buffer Tris·HCl pH 7.5 (200 μL). Ellman's reagent (10 mM in acetonitrile, 0.6 eq) was added, and the resulting mixture was stirred for 1 h. The reaction was quenched using 0.1 % TFA (aq), and right after checking by HPLC-MS the formation of the desired product the crude was purified by reverse phase HPLC. Following characterization, the collected fractions were freeze-dried and stored at −20 ºC. The monomeric disulfide peptide **[AQ]MYC(S-S)** (1.3 mg, 69 % from pure starting peptide) was obtained as a white solid.

### [AQ]MYC(S-S)

$R_t$ = 17 min (column Eclipse XDB-$C_{18}$, lineal gradient 5 to 95 % $CH_3CN$, 0.1 % TFA / $H_2O$, 0.1 % TFA in 30 min).

MALDI: [MH]+ calculated for $C_{435}H_{737}N_{137}O_{124}S_2$ = 9934.64, found 9936.5.

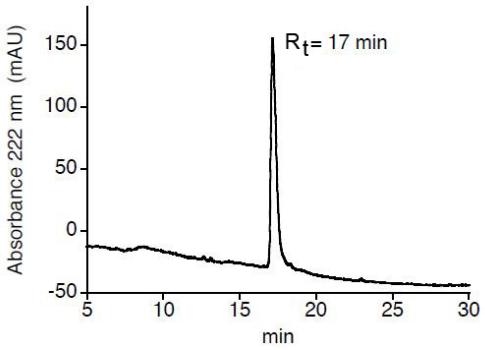
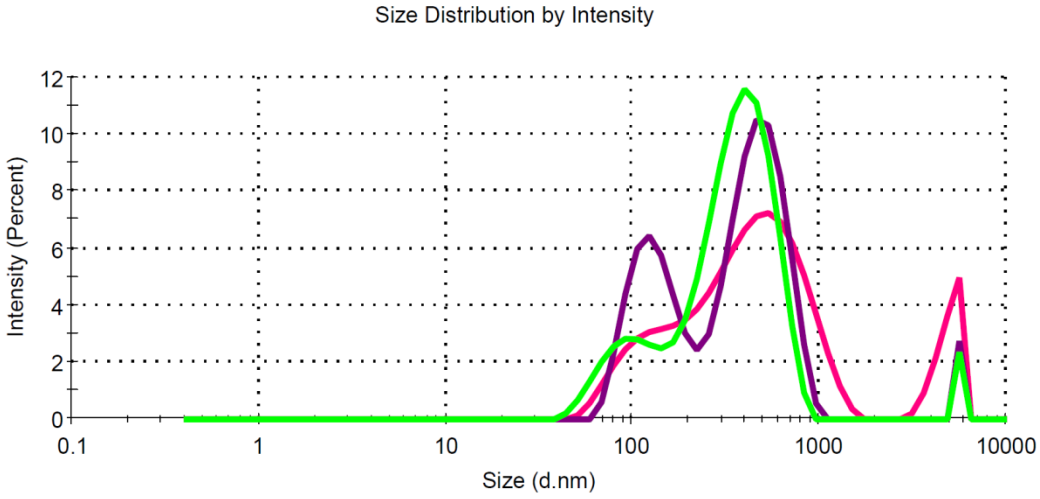



**Figure S28.** HPLC of the pure peptide at 222 nm.

## 12. DYNAMIC LIGHT SCATTERING EXPERIMENTS

To further characterize **[AQ]MYC** and check its physical behavior in solution compared to the **$^{wt}$MYC**, DLS measurements were performed. A *Malvern Nano ZS (Malvern Instruments, U.K.)* was used, operating at 633 nm with a dispersion angle of 173 degrees, attenuator 10 and using a 2 mm light pass cuvette. Solutions of 2 μM of the corresponding peptide in 10 mM Tris·HCl pH 7.5 buffer were measured. Experiments were run at 25 ºC.

a)




B)

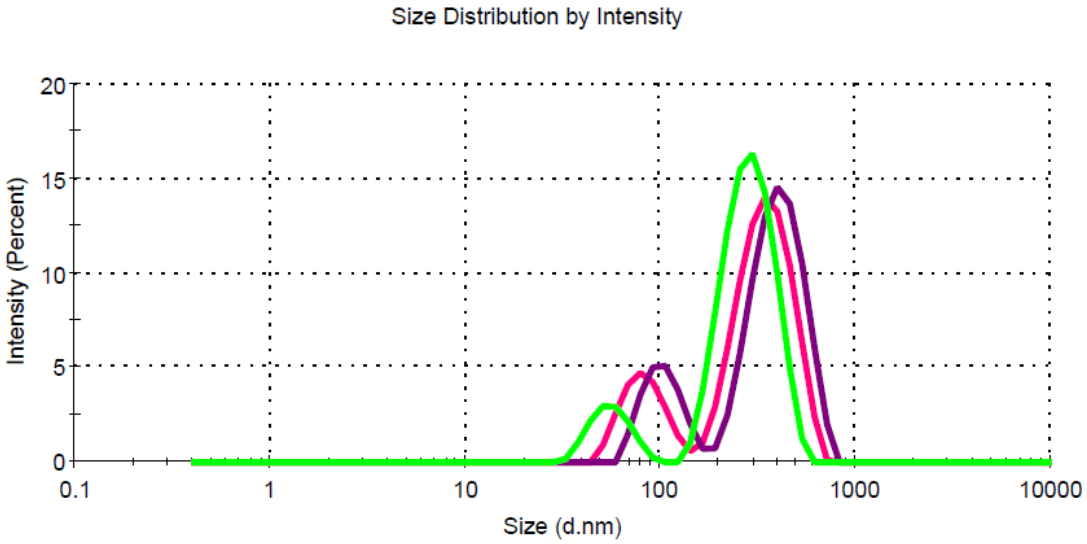



**Figure S29. A)** Overlapping of three size distribution graphs by intensity of a 2 μM solution of the natural **$^{wt}$MYC** protein **B)** Overlapping of three size distribution graphs by intensity of a 2 μM solution of synthetic **[AQ]MYC**.

Although the population distributions are rather similar in both samples, DLS shows that the natural **$^{wt}$MYC** protein forms less defined distributions and presents a significant population of large particles (> 1000 d.nm), consistent with aggregation, that are not observed in the case of **[AQ]MYC**.

## 13. EMSA EXPERIMENTS

EMSA (electrophoretic mobility shift) was performed with a BIO-RAD *Mini Protean* gel system, powered by an electrophoresis power supply *PowerPac*$^{TM}$ Basic model, máximum power 150 V, frequency 50/60 Hz at 140 V (constant V).

In our experiments non-denaturing 10% polyacrylamide gels were used. The preparation of minigels was carried out as follows: in an Erlenmeyer flask 5.95 mL of $H_2O$, 1.05 mL of TBE 5 x, 3.5 mL of protogel (acrylamide: bisacrylamide mixture 30/0.8), 7.5 µL of TMEDA (*N,N,N',N'*-tetramethylethylendiamine) -for radical stabilizing and polymerization speed controlling- and 75 µL of 10% ammonia persulfate -that acts as initiator agent of the radical reaction- are mixed. The mixture is slowly shaken and then is introduced between the plates, placing in last place the shape to form the wells, in which the samples will be loaded. The mixture was allowed to polymerize for 30 min, then the shape was removed and the wells were washed with Milli-Q water. The TBE 5x composition is: 0.445 M TrizmaR base, 0.445 M boric acid, 10 mM EDTA (pH = 8.0).

Binding reactions were performed over 20 min in 18 mM Tris HCl (pH 7.5), 90mM KCl, 1.8 mM $Mg_2Cl$, 1.8 mM EDTA, 9 % glycerol, 0.11 mg/mL BSA and 2.2% NP-40. In the experiments we used 50 nM of the unlabeled ds−DNAs. After incubation for 20 min products were resolved by PAGE using a 10 % non-denaturing poliacrylamide gel and 0.5x TBE buffer for 55 min at 8 ºC and analysed by staining with SYBR$^{TM}$ Gold (from *Molecular Probes*. 5µL in 50 mL of 1x TBE) for 10 min. And visualized by fluorescence 5 x TBE buffer (0.445 M Tris, 0.445 M Boric acid, 10 mM EDTA pH 8.0).

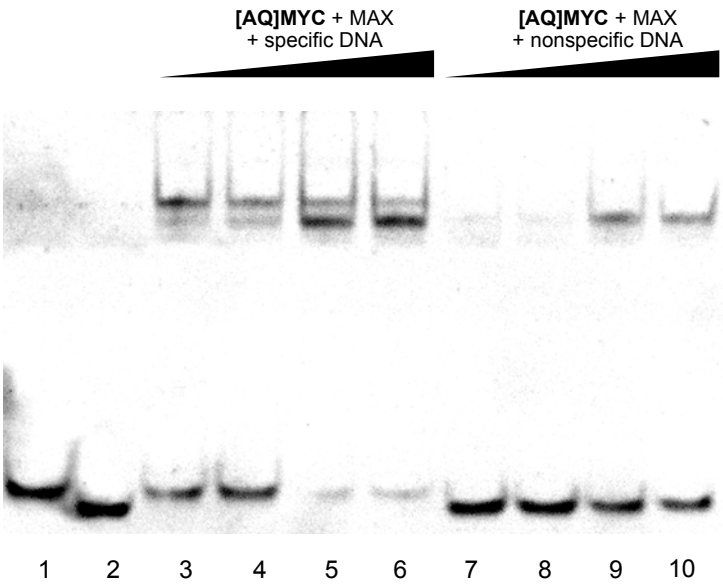



**Figure S30.** EMSA showing the binding of **[AQ]MYC**/ Max complex to a double strand oligonucleotide spanning the E box sequence (specific DNA, **E-DNA**: 5'- CTGTAGGCCACGTGACCGGATG -3'–only one strand is shown–, lanes 1 and 3-6) and to a double strand oligo with a mutated E box sequence (5'-AACGAGATGAGTCATCTCCA - 3'–only one strand is shown–, lanes 2 and 7-10). Lanes 3-6: **[AQ]MYC** concentrations of 150, 300, 500 and 700 nM respectively; and a fixed concentration of MAX of 300 nM in all the lanes; lanes 7-10: **[AQ]MYC** concentrations of 150, 300, 500 and 700 nM, respectively; and a fixed concentration of MAX of 300 nM in all the lanes. All lanes contain 50 nM of DNA.

## 14. CELL UPTAKE EXPERIMENTS

The experiments were carried out at room temperature on a sterile *Tesltar AV-100 bench*. **HeLa** and **A459** cells were cultured in DMEM (Dulbecco Modified Eagle's Medium) containing 10 % of FBS (Fetal Bovine Serum) with 5 mM of L-glutamine and 1 % of Penicillin-Streptomycin-Glutamine Mix. The day before the cellular uptake experiments, cells were seeded in 24 well plates containing glass bottom dishes (15 mm).

Stock solutions of the peptides were dissolved in DMEM without FBS.

For the cell uptake experiments, cells were first washed 3 times with PBS and overlaid with 500 µL of fresh DMEM without serum. Samples with the indicated concentration of the peptide were added, and the mixtures were incubated in an incubator at 37 ºC; with 5 % $CO_2$ atmosphere. Then the medium was removed and the cells were washed with PBS (2 × 1 mL) and fresh DMEM (3 × 1 mL).

Digital pictures were captured with a *Nikon Ti-E microscope* equipped with an *Andor Zyla 4.2 digital camera*. Confocal pictures were taken with an *Andor Dragonfly High Speed Confocal Platform* under identical conditions of gain and exposure for all the samples. Images were further processed (cropping, resizing and global contrast and brightness adjustment) using *Image J* software.

The parameters for the fluorescent channels at the Nikon (Semrock) equipment were:

filter cube DAPI-1160B-000: BP 387/11 nm, LP 447/60 nm and DM 409 nm.
filter cube FITC-3540C-000: BP 482/35 nm, LP 536/40 nm and DM 506 nm.
filter cube TRITCB-000: BP 543/22 nm, LP 593/40 nm and DM 562 nm.

The parameters for the confocal equipment were:

DAPI-LP 450/50 nm and DM 418 nm. $\lambda_{exc}$= 405 nm
Cy3-LP 620/60 nm and DM 567 nm. $\lambda_{exc}$= 561 nm.

When necessary, co-staining experiments were performed to clarify the intracellular distribution of the peptides. **HOECHST 33342** (*Sigma-Aldrich*) was used as a *bona fide* DNA fluorescent probe.

### 14.1. Incubation concentration studies

The appropriate concentration for incubation of the peptides *TMR*-**[AQ]MYC** and *TMR*-**[AQ]MYC(SH)** was studied in HeLa cells for 30 min. Both tagged proteins internalize at nanomolar concentrations. For 50 nM the exposure time and laser intensity had to be prolonged to clearly detect the proteins. At micromolar range the peptides aggregate compromising the cell integrity. So, the best concentration was found to be 250 nM.

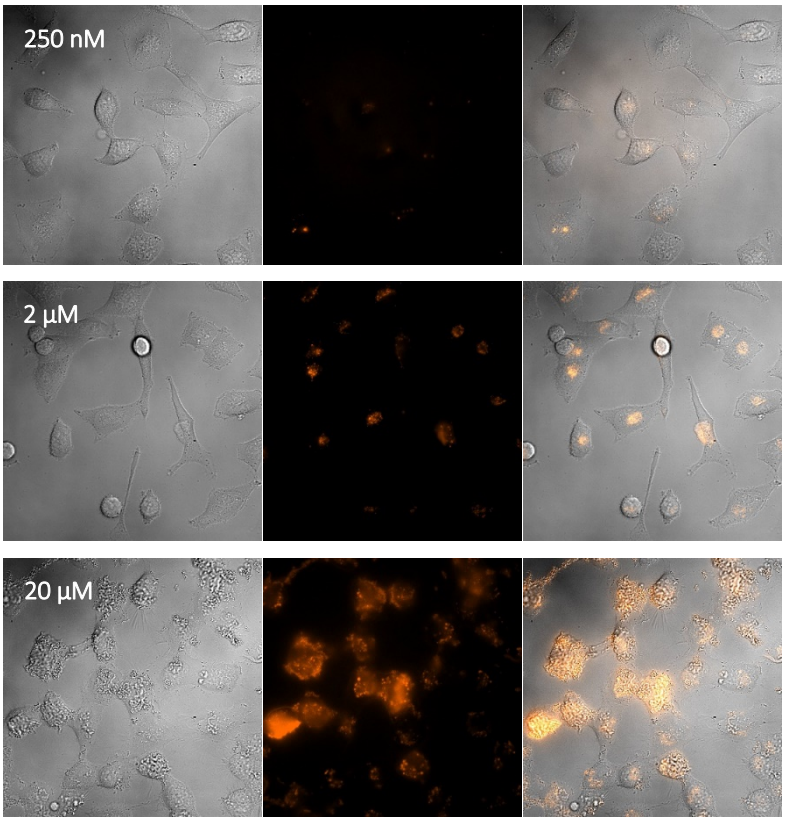



**Figure S31.** HeLa cells incubated with different concentrations of *TMR*-**[AQ]MYC** for 30 min in DMEM without FBS. DIC (Differential Interference Contrast), red channel and overlapping of both DIC and red channels are shown.

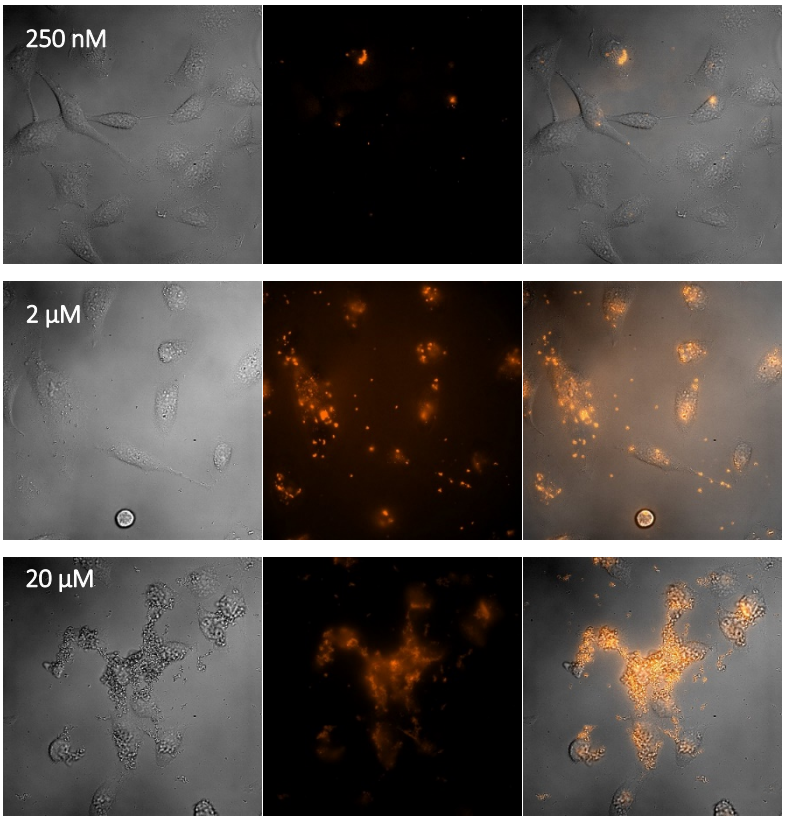



**Figure S32.** HeLa cells incubated with different concentrations of *TMR*-**[AQ]MYC(SH)** for 30 min in DMEM without FBS. DIC, red channel and overlapping of both DIC and red channels are shown

### 14.2. Incubation time

Incubation time was optimized in consequent experiments, once the 250 nM concentration was found to be the best one. We could observe that after 2 h of cell culture using the peptide solutions internalization is taking place. On the other hand, around 4 h is enough for the peptides to completely get inside the cell. We have also observed 24 h is too much time, as some degradation of the peptides might be occurring.

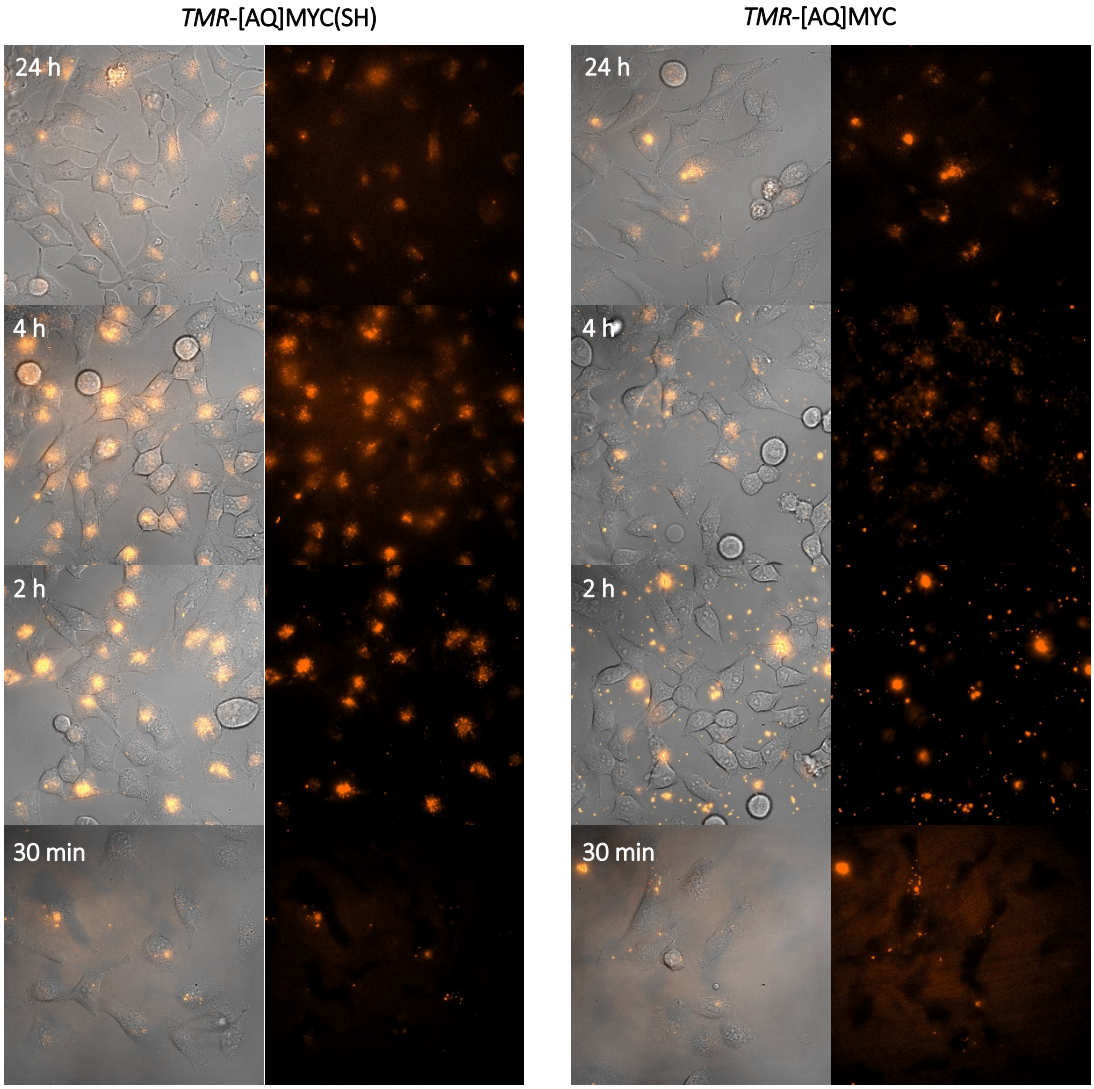



**Figure S33.** Comparison of different incubation times of the two MYC derivatives (*TMR*-**[AQ]MYC(SH)**, left; *TMR*-**[AQ]MYC**, right) in HeLa cells at 250 nM. Incubations were performed in DMEM solutions without FBS. Then, cells were washed twice with fresh DMEM and visualized under the microscope.

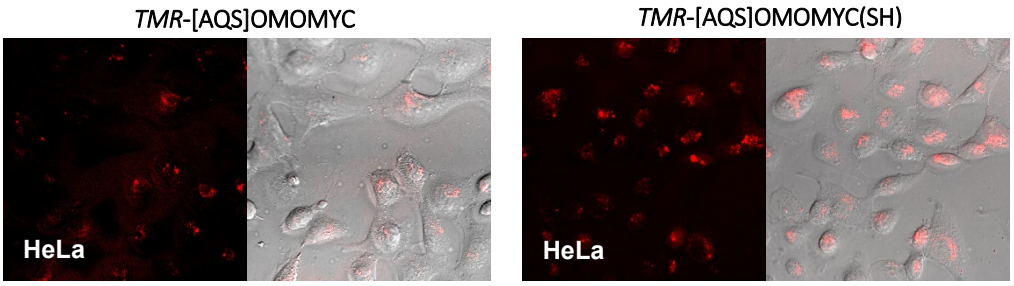



**Figure S34.** OMOMYC derivatives internalized by HeLa cells. 250 nM of the labelled peptides were incubated for 4 h in DMEM without FBS. Then, cells were washed twice with PBS and three more times with fresh DMEM before pictures were taken. Red channel and merge of DIC and red channel is shown.

## 14.3. Internalization by diferent cell lines, localization and comparison of the internalized amounts

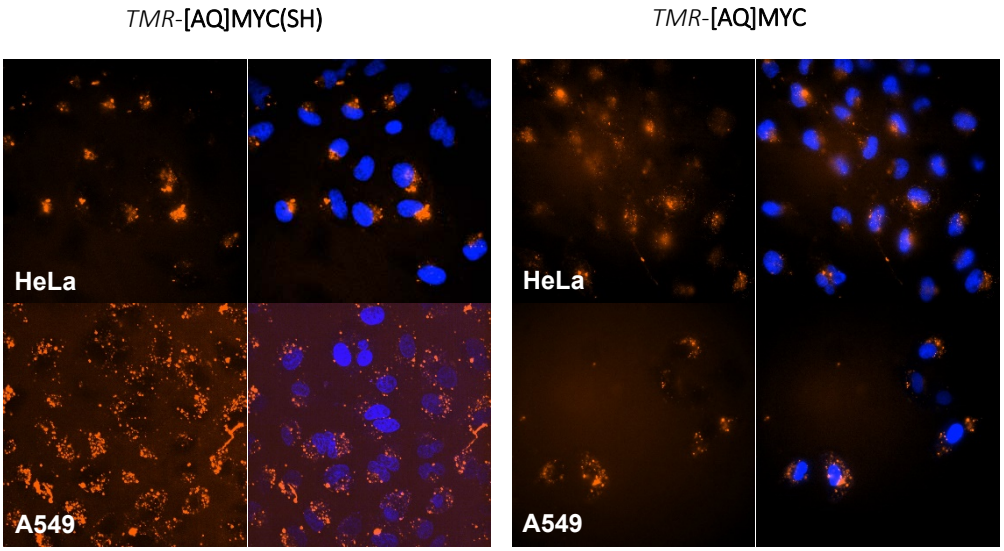



**Figure S35.** HeLa (top row) and A549 (bottom row) cells incubated with 250 nM of the tagged peptides in DMEM for 4 h. Nuclei tagged with HOESCHT 10 μM for 15 min before washing. Red channel and merge of blue and red channel is shown.

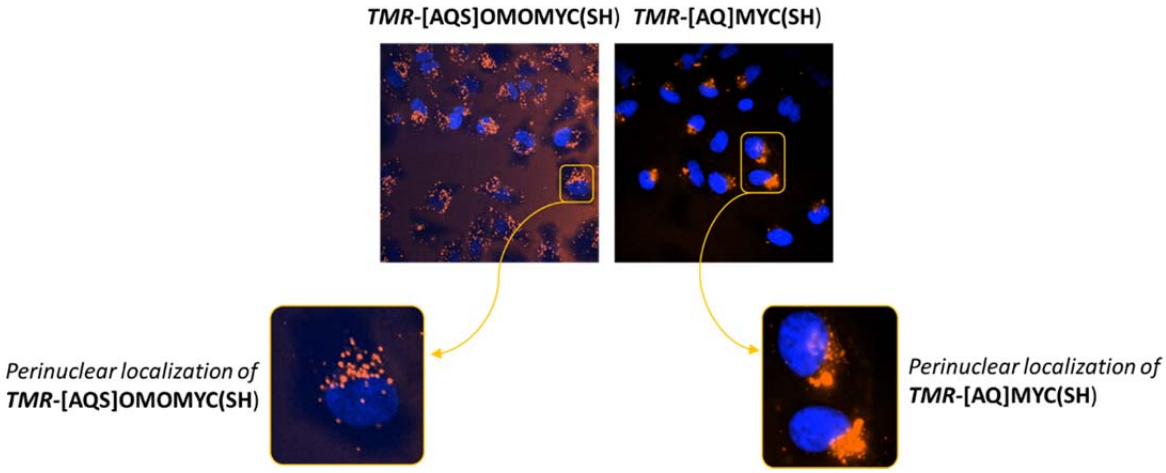



**Figure S36.** Incubation the cells with a nuclei stain allowed us to determine that the peptides are mainly trapped in vesicles around the nuclei in both MYC and OMOMYC cases as we can see in the images above where a zoom of a region of the plate was done.

To perform the comparative analysis, the background noise values were subtracted from the data, so only foreground pixels of the signals were measured to give a relative estimation ratio of penetration between the peptides containing *Cys* residues with those lacking them.

In A549 cells, *TMR*-**[AQS]OMOMYC(SH)** penetrates around 4 times better than its analogue without SH. In addition, *TMR*-**[AQ]MYC(SH)** 3 times better than *TMR*-**[AQ]MYC**.

In HeLa cells, *TMR*-**[AQS]OMOMYC(SH)** penetrates around 7 times better than its analogue without SH. In addition, *TMR*-**[AQ]MYC(SH)** internalizes from 7 to 8 times better than *TMR*-**[AQ]MYC**.

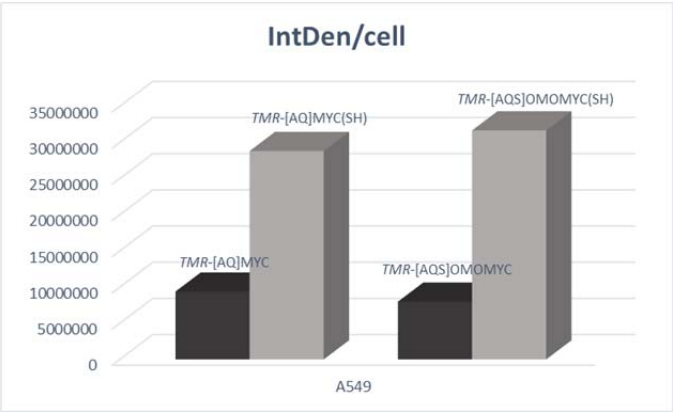



**Figure S37.** Quantification of labelled-protein uptake by A549 cells, by comparison of the integrated fluorescent densities of each protein per cell.

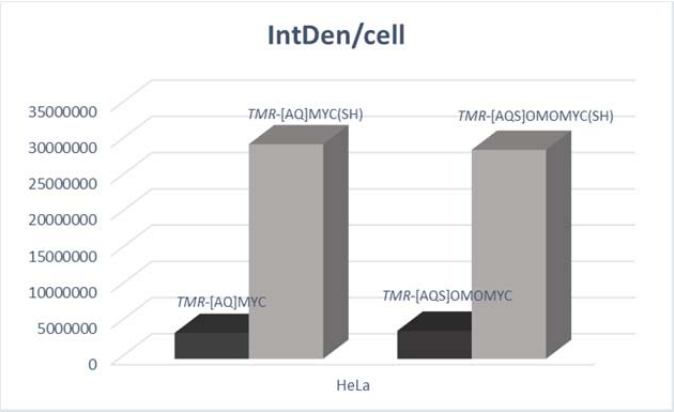



**Figure S38.** Quantification of labelled-protein uptake by comparison of the integrated fluorescent densities of each protein per cell in HeLa cells.

## 15. CIRCULAR DICHROISM

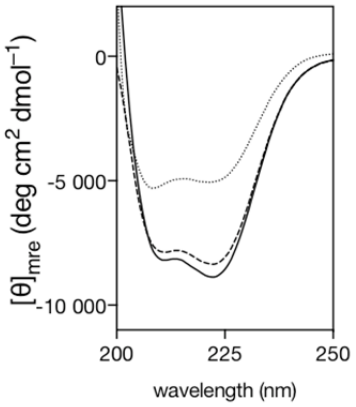



**Figure S39.** Circular dichroism of a 5 μM solution of the peptide MAX (dotted line) in 10 mM phosphate, 150 mM KCl, 1 mM DTT, 0.25 mM EDTA buffer pH 7.4; the same peptide in the presence of 1 equiv. of **[AQ]MYC** (dashed line) and in the presence of 1 equiv. of **[AQ]MYC** peptide and 1 eq of the **E-DNA** (solid line).