Supporting Information

for

Engineering bioactive dimeric transcription factor analogs via palladium rebound reagents

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1. Experimental

1.1 Materials

Fmoc-L-Phe-OH, Fmoc-L-Asn-OH, Fmoc-L-Met-OH, Fmoc-L-Gln-OH, Fmoc-L-Arg(Pbf)-OH, Fmoc-L-Tyr(*t*Bu)-OH, Fmoc-L-Glu(O*t*Bu)-OH, Fmoc-L-Ala-OH, Fmoc-L-Leu-OH, Fmoc-L-His(Trt)-OH, Fmoc-L-Asp-OH, Fmoc-L-Pro-OH, Fmoc-L-Cys(Trt)-OH, Fmoc-L-Lys(Boc)-OH, Fmoc-L-Ile-OH, Fmoc-L-Ser(*t*Bu)-OH, and Fmoc-L-Gly-OH, were purchased from CreoSalus, Inc. (Louisville, KY) or Novabiochem. 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU), was purchased from P3 Biosystems (Louisville, KY). H-Rink Amide ChemMatrix resin was obtained from PCAS BioMatrix Inc. (St-Jean-sur-Richeliue, Quebec, Canada). Oligonucleotides were purchased from Integrated DNA Technologies (IDT, Coralville, IA). . Diethyl ether (Et_2O, ≥98% stabilized, ACS grade), dichloromethane (CH_2Cl_2, (≥99.5% stabilized ACS) were obtained from VWR International (Philadelphia, PA). Synthesis-grade *N,N*-dimethylformamide (DMF, OmniSolv® and stored with an AldraAmine trapping packet), trifluoroacetic acid (TFA, ≥99% ReagentPlus®), diisopropylethylamine (DIEA, ≥99% ReagentPlus®), piperidine (≥99% ReagentPlus®), triisopropylsilane (TIS, 98%), acetonitrile (ACN, LC/MS Grade), formic acid (FA, 98-100% for LC/MS), and dimethyl sulfoxide (DMSO, ≥99.5% ReagentPlus®) were purchased from MilliporeSigma. The ligand SPhos was generously gifted from MilliporeSigma. Bolt™ 4-12% Bis-Tris Plus Gels, pre-stained Invitrogen SeeBlue Plus2 molecular weight standard, Bolt™ LDS Sample Buffer (4X), Laemmli buffer (4X), and Orange DNA Loading Dye (6X) were purchased from Thermo Fisher Scientific. Water for all reactions carried out on proteins, used in calibration curves, and for reverse-phase purification was obtained via filtration of deionized water through a MilliporeSigmaTM Milli-Q™ Ultrapure Water System. Amine-free DMF refers to DMF stored over AldraAmine trapping packets. All deuterated solvents were purchased from Cambridge Isotope Laboratories and used without further purification. All other chemicals were obtained from MilliporeSigma and used as received without further purification. Cell lines, HeLa (ATCC CCL-2), A549 (ATCC CCL-185), and H441 (ATCC HTB-174), were obtained from ATCC. Media (MEM, FK-12, and RPMI-1640), fetal bovine serum (FBS), penicillin-streptomycin, and 0.25% trypsin-EDTA were obtained from Gibco. Culture flasks, plates, and serological pipettes were obtained from VWR.

1.2 Liquid-chromatography mass spectrometry (LC-MS) analysis

LC-MS analysis: LC-MS chromatograms and associated mass spectra were acquired using Agilent 6545 or 6550 ESI-QToF mass spectrometer. Mobile phases used for LC-MS analysis are solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile). MS conditions: positive electrospray ionization (ESI) extended dynamic mode in mass range 300–3000 m/z, temperature of drying gas = 350 °C, flow rate of drying gas = 11 L/min, pressure of nebulizer gas = 60 psi, the capillary, fragmentor, and octupole rf voltages were set at 4000, 175, and 750, respectively. The following LC-MS methods were used:

Method A: Agilent 6550 Aeris-C_4 column (2.1 x 150 mm, 3.6 µm), 40 °C, LC conditions: 1% B from 0–2 min, linear gradient from 1% to 91% B from 2–18 min, then 91-95% 18-21 min, 0.4 mL/min flow rate.

Method B: Agilent 6550 Zorbax 300SB-C_3 column (2.1 x 150 mm, 5 µm), 40 °C, LC conditions: 1% B from 0–2 min, linear gradient from 1% to 61% B from 2–12 min, then 91-95% 12-15 min, 0.4 mL/min flow rate.

Method C: Agilent 6550 Zorbax 300SB-C_3 column (2.1 x 150 mm, 5 µm), 40 °C, LC conditions: 1% B from 0–1 min, linear gradient from 1% to 61% B from 1–8 min, then 61-95% 8-9 min, 0.4 mL/min flow rate.

Method D: Agilent 6545 Zorbax 300SB-C_3 column (2.1 x 150 mm, 5 µm), 40 °C, LC conditions: 1% B from 0–2 min, linear gradient from 1% to 91% B from 2–12 min, then 91-95% 12-15 min, 0.8 mL/min flow rate.

Method E: Agilent 6550 Aeris-C_4 column (2.1 x 150 mm, 3.6 µm), 40 °C, LC conditions: 1% B from 0–2 min, linear gradient from 1% to 91% B from 2–12 min, then 91-95% 12-15 min, 0.4 mL/min flow rate.

1.3 Reversed-phase High-pressure liquid-chromatography (RP-HPLC) analysis

Analytical RP-HPLC analysis: RP-HPLC chromatograms were acquired using an Agilent 1200 RP-HPLC. Mobile phases used are solvent A (0.1% trifluoroacetic acid in water) and solvent B (0.1% trifluoroacetic acid in acetonitrile).

Method A: Zorbax 300SB-C3 column (2.1 x 150 mm, 5 µm); LC conditions: 5% B from 0–1.5 min, linear gradient from 5% to 65% B from 1.5–20 min, then 65% to 95% B 20-22 min, then 95% to 5% B 22-25 min, 0.4 mL/min flow rate.

1.4 Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE: The analysis was performed using Bolt™ 4-12% Bis-Tris Plus Gels (10-wells) at 165 V for 36 min utilizing pre-stained Invitrogen SeeBlue™ Plus2 molecular weight standard with Bolt™ LDS Sample Buffer (4X). ~ 1 μg of protein loaded to the gel. The bands were visualized by Coomassie blue staining.

Note: for the analysis of the crude conjugation reactions, samples were incubated at 95 °C for 5 min prior loading into the gel.

1.5 Reversed-phase (RP) HPLC Purification

Reversed-phase preparative HPLC was performed using an Agilent mass directed purification system (1260 Infinity LC and 6130 single quad MS), equipped with an Agilent Zorbax SB-C3 column (9.4 x 250 mm, 5 μm). Mobile phases used for LC-MS analysis were solvent A (0.1% TFA in water), solvent B (0.1% TFA in acetonitrile). The following LC-MS methods were used:

Method A: Zorbax 300SB-C_3 column (9.4 x 250 mm, 5 μm), LC conditions: 5% B from 0–5 min, linear gradient from 20% to 65% B from 5-70 min, 4 mL/min flow rate at 60 °C.

Method b: Zorbax 300SB-C_3 column (9.4 x 250 mm, 5 μm), LC conditions: 5% B from 0–5 min, linear gradient from 20% to 65% B from 5-70 min, 4 mL/min flow rate.

1.6 Reverse phase (RP) flash chromatography purification

The CombiFlash® flash chromatography system was used to purify specified monomers by reverse phase (RP) chromatography. The purifications was performed using 25 g Biotage Sfär Bio C18 D 20 μm column. Mobile phases used for LC-MS analysis were solvent A (0.1% TFA in water), solvent B (0.1% TFA in acetonitrile)

Method A: Using 25 g Sfär Column; gradient conditions: 15% B for 1 CV, linear ramp from 15% to 60% B for 15 CV, linear ramp from 60% to 90% for 1 CV, 40 mL/min flow rate.

1.7 Determination of protein monomers and dimers yield

Monomer analogs: The yield of purified protein (Myc, Max, and Omomyc) was calculated gravimetrically after lyophilization.

Dimer analogs: The yield of isolated dimer (Max-Max, Myc-Myc, Omomyc-Omomyc, Myc-Max, Omomyc-Myc, Max-Omomyc, and TAMRA-Max-Max) were calculated via the UV-Vis absorbance at 280nm. Each isolated analog was dissolved in DMSO, and the concentration of the target analog was calculated using the Beer-Lambert law by measuring the absorbance of the protein sample at 280 nm (see section 1.7). The absorbance was measured by averaging four independent readings of the same sample on

a BioTek Synergy HT plate reader outfitted with a BioTek Take 2 micro-volume plate. The molar extinction coefficient of the protein of interest was estimated based on the sequence of the protein via using AAT Bioquest Inc. software (see section 1.7).

1.8 Determination of protein concentrations

The concentration of proteins were determined by using the molecular weight, molar extinction coefficient, and absorbance at λ_{max} with the Beer-Lambert Law.

$$\text{Concentration} = \frac{\text{Absorbance at } \lambda_{max}}{\text{Extinction coefficient} \times \text{Pathlength}} \times \text{Molecular weight}$$

The absorbance at λ_{max} was measured using a Biotek Epoch microplate reader at 280 nm. Extinction coefficient of the proteins were calculated using AAT Bioquest Inc. software. The following are the molar extinction coefficients used in this work:

Max: 2680 M^{-1} cm^{-1}

Myc: 1400 M^{-1} cm^{-1}

Omomyc: 1400 M^{-1} cm^{-1}

S-aryl linkage as previously reported[1]: 14,400 $M^{-1}cm^{-1}$

Max-Max: 19760 M^{-1} cm^{-1}

Myc-Myc: 17200 M^{-1} cm^{-1}

Omomyc-Omomyc: 17200 M^{-1} cm^{-1}

Myc-Max: 18480 M^{-1} cm^{-1}

Myc-Omomyc: 17200 M^{-1} cm^{-1}

Max-Omomyc: 18480 M^{-1} cm^{-1}

TAMRA-Max-Max: 43760 M^{-1} cm^{-1}

2. Synthesis of bifunctional palladium(II) OAC

Synthesis of sSPhos. This compound was prepared according to literature procedure.[1] The ^{1}H and ^{13}C NMR spectra of the obtained material are identical to those reported in the literature.

Synthesis of [(cod)Pd($CH_2TMS)_2$]. This compound was prepared according to literature procedure.[2] The ^{1}H and ^{13}C NMR spectra of the obtained material are identical to those reported in the literature.

Synthesis of Pd OAC 4. This compound was prepared according to literature procedure.[1] The ^{1}H and ^{13}C NMR spectra of the obtained material are identical to those reported in the literature.

3. Peptide synthesis

3.1 General procedure for automated flow peptide synthesis

The three proteins (Max **1**, Myc **2**, and Omomyc **3**) were synthesized on an automated flow-based SPPS system (Amidator) as previously reported.[3] The synthesis carried out utilizing standard protocol using 100 mg resin ChemMatrix® Rink amide resin (loading 0.18 mmol/g, typical scale: 100 mg, 18 μmol) was loaded into a fritted syringe (12 mL), swollen in DMF (5 mL) and loaded into the Amidator reactor. The following settings were used for protein synthesis: flow-rate = 40 mL/min, temperature = 90 °C (loop) and 85–90 °C (reactor). The 50 ml/min pump head pumps 400 μL of liquid per pump stroke; the 5 mL/min pump head pumps 40 μL of liquid per pump stroke. The standard synthetic cycle involves a first step of prewashing the resin at elevated temperatures for 60 s at 40 mL/min. During the coupling step, three HPLC pumps are used: a 50 mL/min pump head pumps the activating agent, a second 50 ml/min pump head pumps the amino acid and a 5 mL/min pump head pumps DIEA. The coupling–deprotection cycle is repeated for all additional monomers. Following the synthesis, the resin washed 3 times with CH_2Cl_2 and dried.

3.2 General procedure for TAMRA labeling

TAMRA labeled proteins were synthesized using the flow-based SPPS method as described in section **3.1**. Next, target protein was coupled manually with carboxytetramethylrhodamine (1.0 mmol) in HATU (2.5 mL, 0.38 M in DMF) followed by the addition of DIEA (0.5 mL). The solution was allowed to sit for 1 min at room temperature, and then was added to the resin by syringe. The solution was incubated and reacted with the resin for 15 min with stirring at 5 min increments with a metal spatula. After completion of the coupling step, the coupling solution was evacuated, and the syringe was washed with DMF (3 x 10 mL). Upon completion of the synthesis, the resin was washed with CH_2C_{l2} (3x), dried under high vacuum.

3.3 General procedure for peptide cleavage

Each peptide was subjected to global sidechain deprotection and cleavage from resin by treatment with 5.0-10.0 mL of a solution containing 82.5% TFA, 5% water, 5% phenol, 5% thioanisole, 2.5% 1,2-Ethanedithiol for 3 hours at room temperature. The cleavage mixture was filtered through a fritted syringe, and the

combined filtrate was added dropwise to Et_2O (40 mL) in a separate conical tube that had been chilled for 30 min on dry ice. The resulting precipitate was pelleted by centrifugation (3220 rcf for 5 min), and the supernatant was discarded. Additional Et_2O chilled on dry ice was added to the conical tube which was vortexed to break up the pellet. The precipitate was pelleted by centrifugation, the supernatant was discarded, and the process of Et_2O addition, vortexing, and pelleting was repeated once more. The precipitated crude product pellet was dissolved in 50% (v/v) acetonitrile and water, flash-frozen in liquid nitrogen, and lyophilized for 24 h. Myc, Max, and Omomyc were purified via RP-flash chromatography using Method A as described in section 1.6.

4. DNA and protein sequences

Max (1):

DKRAHHNALERKRRDHIKDSFHSLRDSVPSLQGEKASRAQILDKATEYIQYMRRKNHTHQQDID
DLKRQNALLEQQVRALGGC

Myc (2):

NVKRRTHNVLERQRRNELKRSFFALRDQIPELENNEKAPKVVILKKATAYILSVQAEEQKLISEE
DLLRKRREQLKHKLEQLGGC

Omomyc (3):

MATEENVKRRTHNVLERQRRNELKRSFFALRDQIPELENNEKAPKVVILKKATAYILSVQAETQ
KLISEIDLLRKQNEQLKHKLEQLRNSC

E-box DNA probe:

5’-CCGGCTGA**CACGTG**GTATTAAT-3’

5. Chemical synthesis of protein monomers

5.1 Max (1)

Max was synthesized via automated fast-flow SPPS as described in section 3.1. 14.4 μmol of the resin was cleaved and lyophilized as described in section 3.3. The protein was obtained as lyophilized powder (115 mg). The crude protein was purified via RP-flash chromatography using method A to obtain the purified Myc in 44 % isolated yield (50.5 mg).

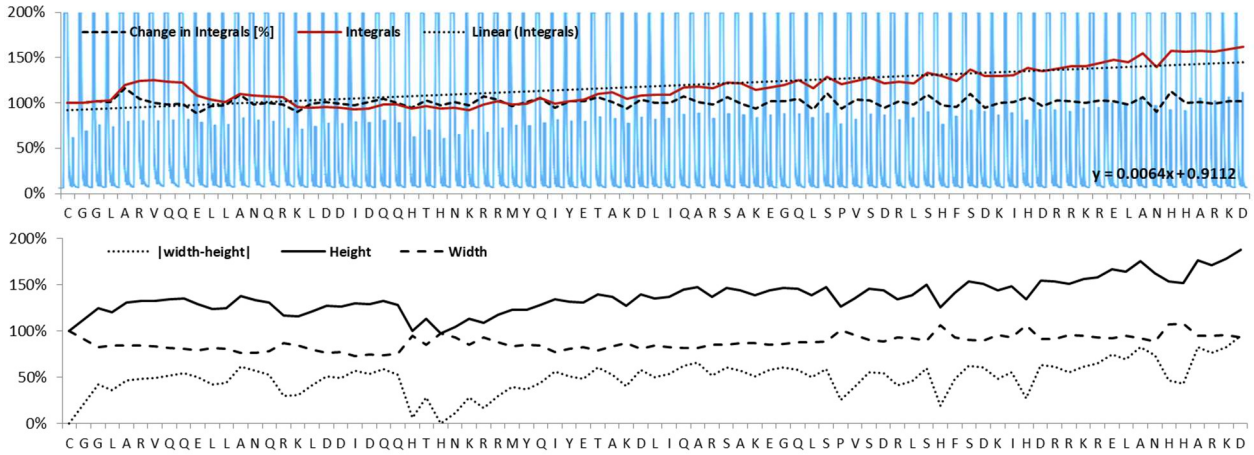


Figure S1. In-line UV_{310nm} monitoring for Fmoc-deprotection for Max (1). Shown are the absorption traces during the synthesis (grey – upper inset). Each peak corresponds to an Fmoc deprotection step and indicates the coupling efficiency of each synthesis step. The corresponding integrals are shown as a red line in the upper inset. In the bottom inlet the peak width and height are shown separately. The deflections with lowered peak height and broader peak width correspond to histidine incorporations, which were performed at 70 °C instead of 90 °C. Synthesis time: 3.4 hours.

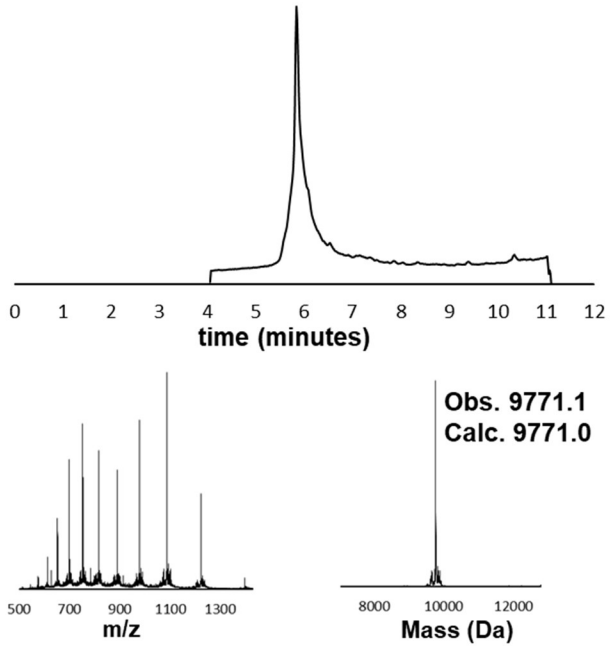


Figure S2. LC-MS analysis of Max 1 (crude). Total ion current chromatogram (TIC) and the m/z and deconvoluted mass spectrum of **1**. LC-MS analysis was carried out using Method D (see section 1.2).

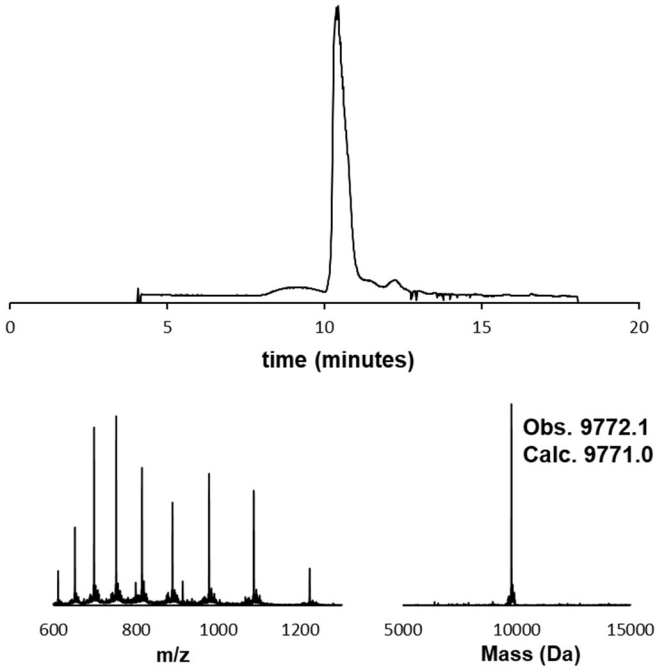


Figure S3. LC-MS analysis of Max 1 (pure). Total ion current chromatogram (TIC) and the m/z and deconvoluted mass spectrum of **1**. LC-MS analysis was carried out using Method A (see section 1.2).

5.2 Myc (2)

Myc was synthesized via automated fast-flow SPPS as described in section 3.1. 14.4 μmol of the resin was cleaved and lyophilized as described in section 3.3. The protein was obtained as lyophilized powder (89 mg). 45 mg crude protein was purified via RP-flash chromatography using method A to obtain the purified Myc in 38 % isolated yield (17.2 mg).

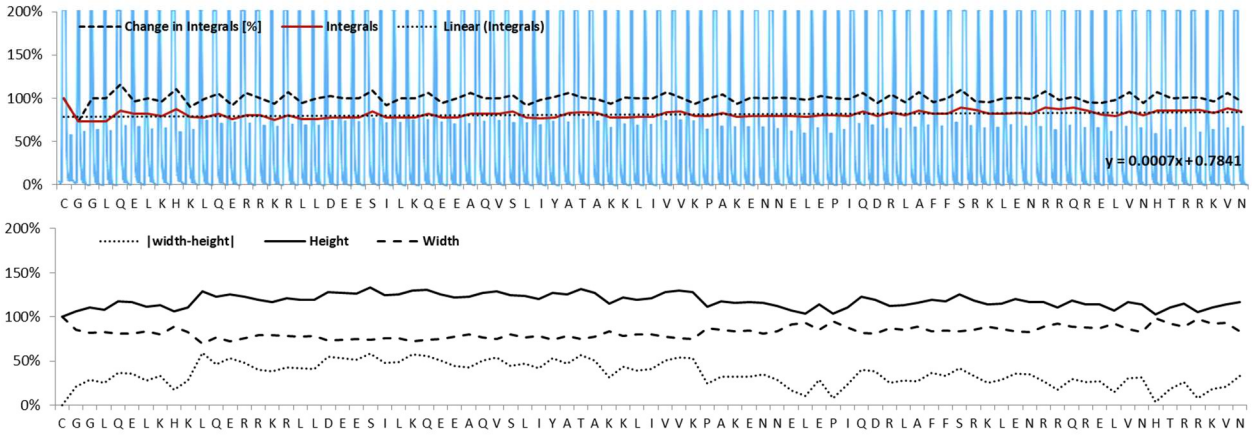


Figure S4. In-line UV_{310nm} monitoring for Fmoc-deprotection for Myc (2). Shown are the absorption traces during the synthesis (grey – upper inset). Each peak corresponds to an Fmoc deprotection step and indicates the coupling efficiency of each synthesis step. The corresponding integrals are shown as a red line in the upper inset. In the bottom inlet the peak width and height are shown separately. The deflections with lowered peak height and broader peak width correspond to histidine incorporations, which were performed at 70 °C instead of 90 °C. Synthesis time: 3.5 hours.

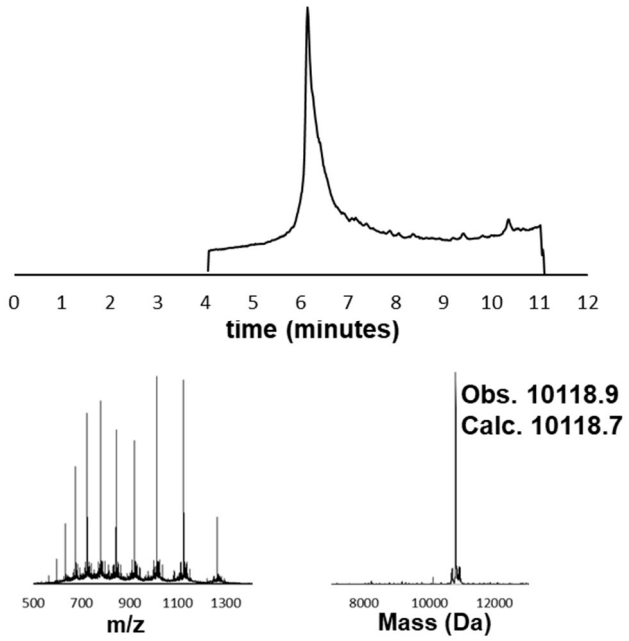


Figure S5. LC-MS analysis of Myc 2 (crude). Total ion current chromatogram (TIC), the mass-to-charge (m/z) spectrum, and deconvoluted mass spectrum of **2**. LC-MS analysis was carried out using Method D (see section 1.2).

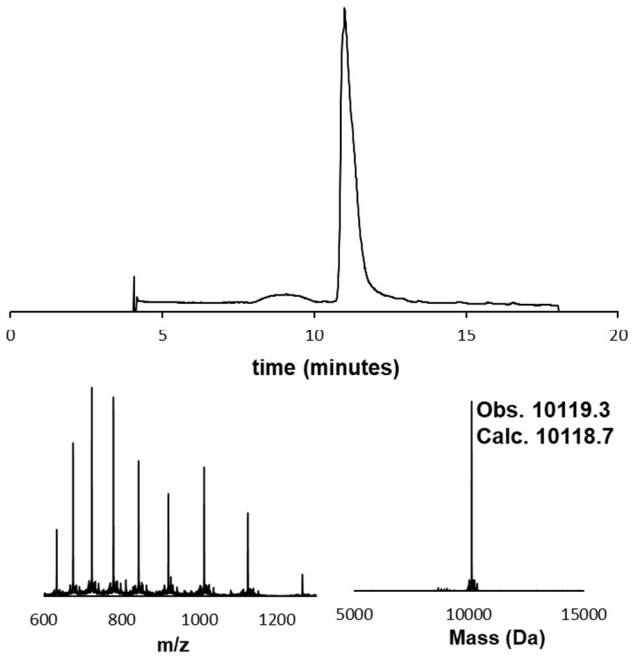


Figure S6. LC-MS analysis of Myc 2 (pure). Total ion current chromatogram (TIC), the mass-to-charge (m/z) spectrum, and deconvoluted mass spectrum of **2**. LC-MS analysis was carried out using Method A (see section 1.2).

5.3 Omomyc (3)

Omomyc was synthesized via automated fast-flow SPPS as described in section 3.1. 14.4 μmol of the resin was cleaved and lyophilized as described in section 3.3. The protein was obtained as lyophilized powder (118 mg). 70 mg crude protein was purified via RP-flash chromatography using method A to obtain the purified Myc in 40% isolated yield (28.1 mg).

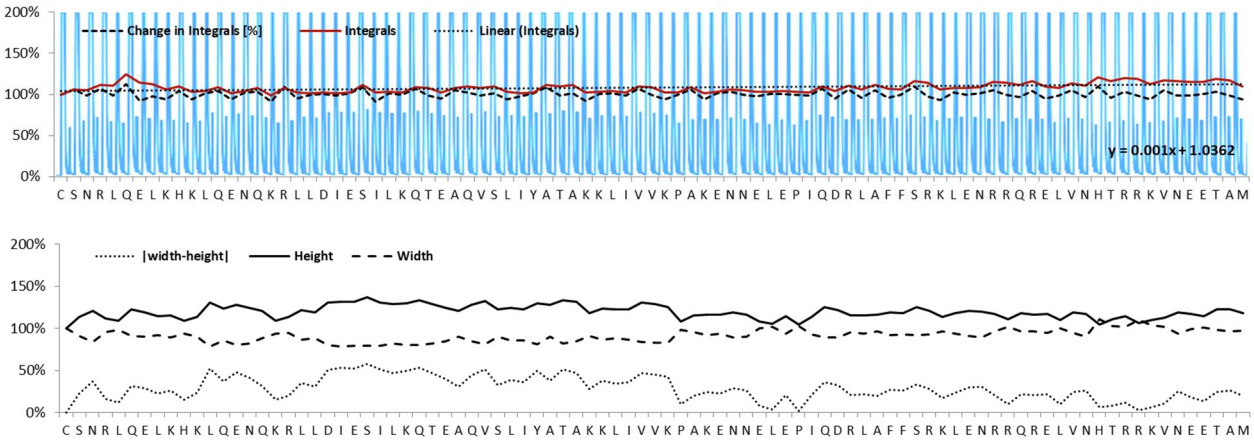


Figure S7. In-line UV_{310nm} monitoring for Fmoc-deprotection for Omomyc (3). Shown are the absorption traces during the synthesis (grey – upper inset). Each peak corresponds to an Fmoc deprotection step and indicates the coupling efficiency of each synthesis step. The corresponding integrals are shown as a red line in the upper inset. In the bottom inlet the peak width and height are shown separately. The deflections with lowered peak height and broader peak width correspond to histidine incorporations, which were performed at 70 °C instead of 90 °C. Synthesis time: 3.7 hours.

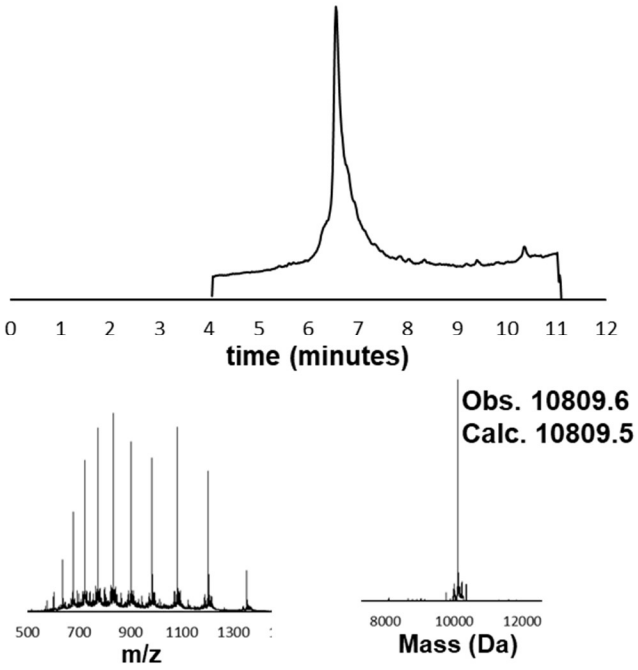


Figure S8. LC-MS analysis of Omomyc 3 (crude). Total ion current chromatogram (TIC), mass-to-charge (m/z) spectrum, and deconvoluted mass spectrum of **3**. LC-MS analysis was carried out using Method D (see section 1.2).

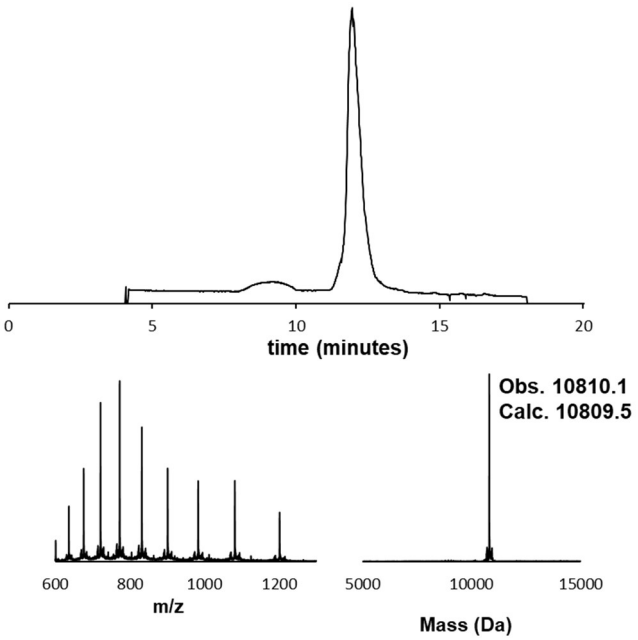


Figure S9. LC-MS analysis of Omomyc 3 (pure). Total ion current chromatogram (TIC), mass-to-charge (m/z) spectrum, and deconvoluted mass spectrum of **3**. LC-MS analysis was carried out using Method A (see section 1.2).

5.4 TAMRA-Max (11)

TAMRA-Max was synthesized via automated fast-flow SPPS described in section 3.2. 3.6 μmol of the resin was cleaved and lyophilized as described in section 3.3. The protein was obtained as lyophilized powder (25 mg). 25 mg crude protein was purified via RP-flash chromatography using method A to obtain the purified TAMRA-Omomyc in 40% isolated yield (9.5 mg).

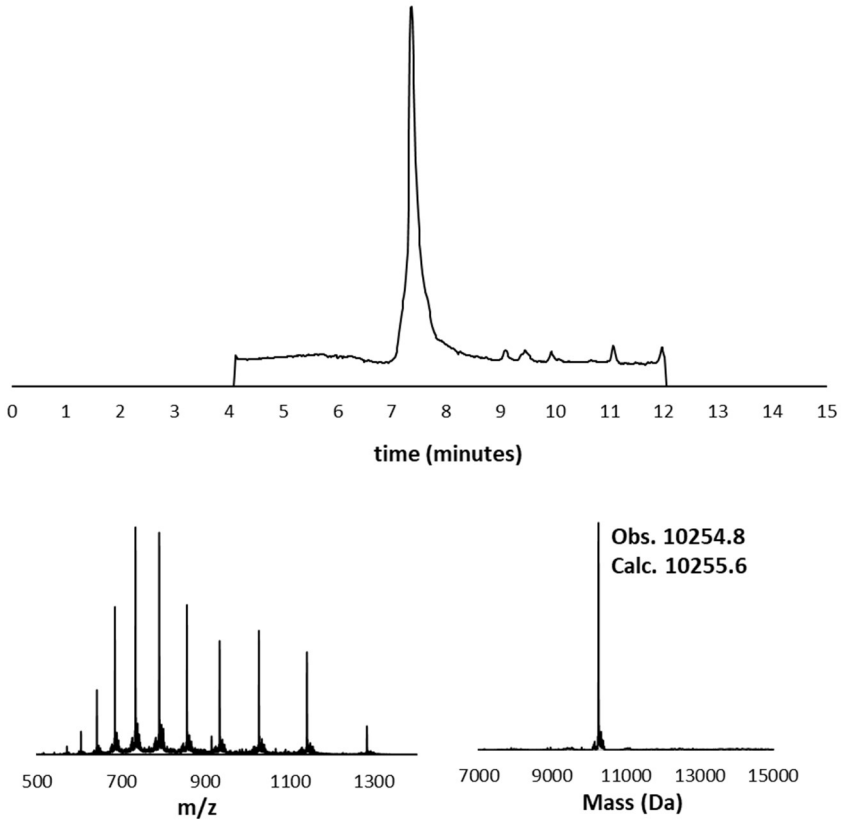
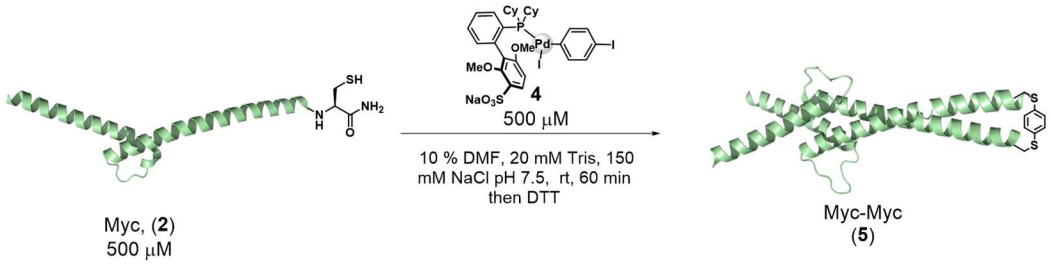


Figure S10. LC-MS analysis of TAMRA-Max 1t (pure). Total ion current chromatogram (TIC), mass-to-charge (m/z) spectrum, and deconvoluted mass spectrum of **1t**. LC-MS analysis was carried out using Method D (see section 1.2).

6. Synthesis of the homodimer analogs

6.1 Myc-Myc



To a 1.5 mL Eppendorf tube was added Myc **2** (300 µL, 10.0 mg/mL, 1.0 equiv) as a solution in 20 mM Tris, 150 mM NaCl (pH 7.5), 234 µL 20 mM Tris, 150 mM NaCl (pH 7.5), 30.5 µL DMF and Pd OAC **4** (28.5 µL, 10.0 mg/mL, 1.0 equiv) as a solution in DMF (titrated over one minute). The final reaction concentration of the major reaction components were the following: **2** (500 µM); **4** (500 µM). The Eppendorf tube was closed, vortexed, and incubated at room temperature for 60 min. A small aliquot was taken from the reaction mixture for analysis by SDS-PAGE (Figure S14). Finally, the reaction was quenched by DTT (10 µl, 1 M in H_2O) and kept at room temperature for 5 min, then purified by RP-HPLC using method A to obtain the purified Myc-Myc in 37% isolated yield.

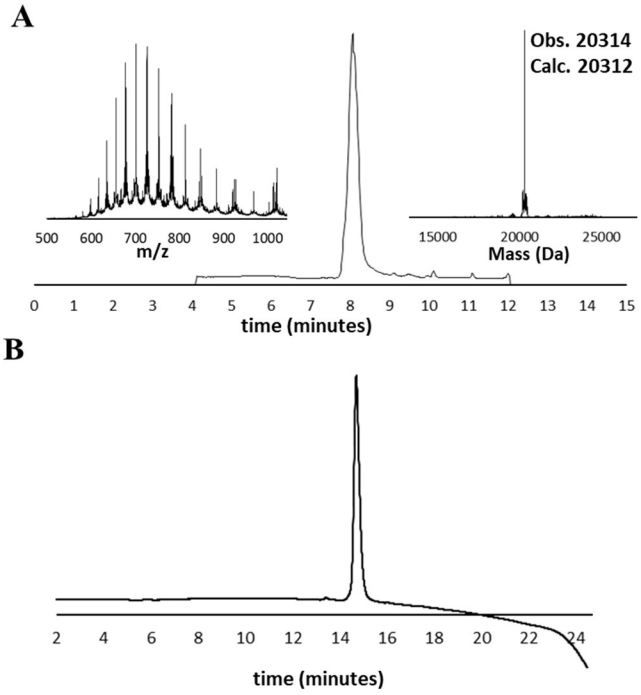
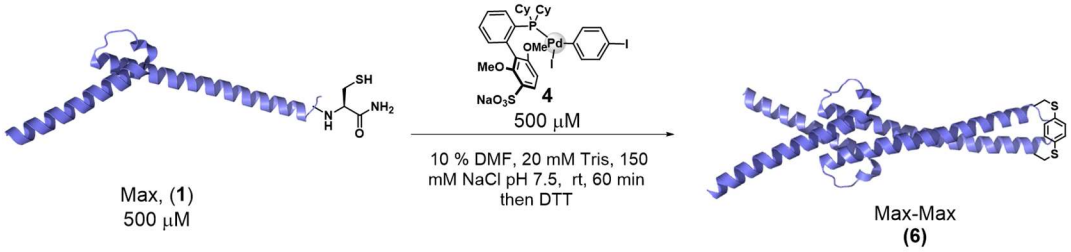


Figure S11. LC-MS and RP-HPLC analysis of purified Myc-Myc (5). A) Total ion current chromatogram (TIC), mass-to-charge (m/z) spectrum, and deconvoluted mass spectrum of **5**. LC-MS analysis was carried out using Method E (see section 1.2). B) HPLC chromatogram of the UV absorbance at 214 nm. HPLC analysis was carried out with Method A (see section 1.3).

6.2 Max-Max



To a 1.5 mL Eppendorf tube was added Max **1** (300 µL, 10.0 mg/mL, 1.0 equiv) as a solution in 20 mM Tris, 150 mM NaCl (pH 7.5), 250 µL 20 mM Tris, 150 mM NaCl (pH 7.5), 31.0 µL DMF and Pd OAC **4** (29.0 µL, 10.0 mg/mL, 1.0 equiv) as a solution in DMF (titrated over one minute). The final reaction concentration of the major reaction components were the following: **1** (500 µM); **4** (500 µM). The Eppendorf tube was closed, vortexed and incubated at room temperature for 60 min. A small aliquot was taken from the reaction mixture for analysis by SDS-PAGE (Figure S14). Finally, the reaction was quenched by DTT (10 µl, 1 M in H_2O) and kept at room temperature for 5 min, then purified by RP-HPLC using method A to obtain the purified Max-Max in 40% isolated yield.

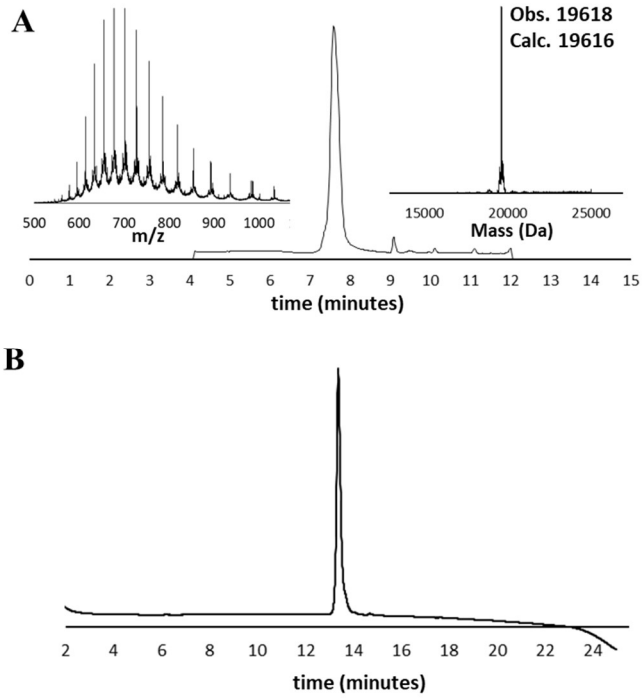
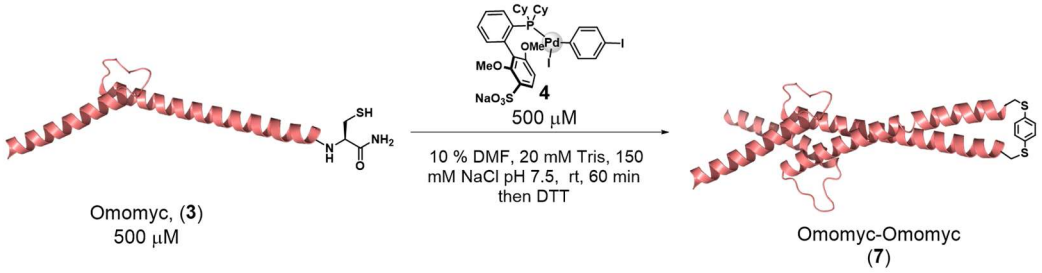


Figure S12. LC-MS and RP-HPLC analysis of purified Max-Max (6). A) Total ion current chromatogram (TIC), mass-to-charge (m/z) spectrum, and deconvoluted mass spectrum of **6**. LC-MS analysis was carried out using Method E (see section 1.2). B) HPLC chromatogram of the UV absorbance at 214 nm. HPLC analysis was carried out with Method A (see section 1.3).

6.3 Omomyc-Omomyc



To a 1.5 mL Eppendorf tube was added Omomyc **3** (300 µL,10.0 mg/mL, 1.0 equiv) as a solution in 20 mM Tris, 150 mM NaCl (pH 7.5), 190 µL 20 mM Tris, 150 mM NaCl (pH 7.5), 26.0 µL DMF and Pd OAC **4** (29.0 µL, 10.0 mg/mL, 1.0 equiv) as a solution in DMF (titrated over one minute). The final reaction concentration of the major reaction components were the following: **3** (500 µM); **4** (500 µM). The Eppendorf tube was closed, vortexed, and incubated at room temperature for 60 min. A small aliquot was taken from the reaction mixture for analysis by SDS-PAGE (Figure S14). Finally, the reaction was quenched by DTT (10 µl, 1 M in H_2O) and kept at room temperature for 5 min, then purified by RP-HPLC using method A to obtain the purified Omomyc-Omomyc in 38% isolated yield.

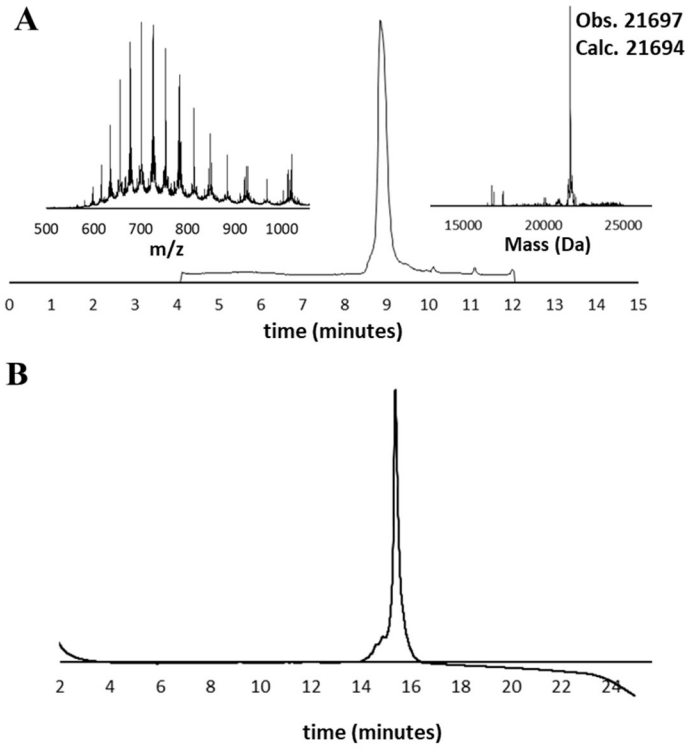


Figure S13. LC-MS and RP-HPLC analysis of purified Omomyc-Omomyc (7). A) Total ion current chromatogram (TIC), mass-to-charge (m/z) spectrum, and deconvoluted mass spectrum of **7**. LC-MS analysis was carried out using Method E (see section 1.2). B) HPLC chromatogram of the UV absorbance at 214 nm. HPLC analysis was carried out with Method A (see section 1.3).

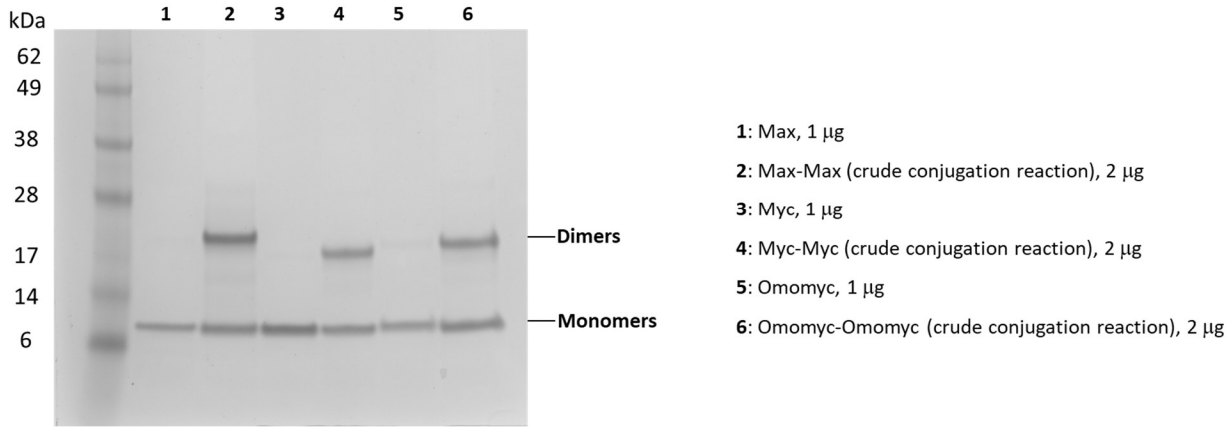
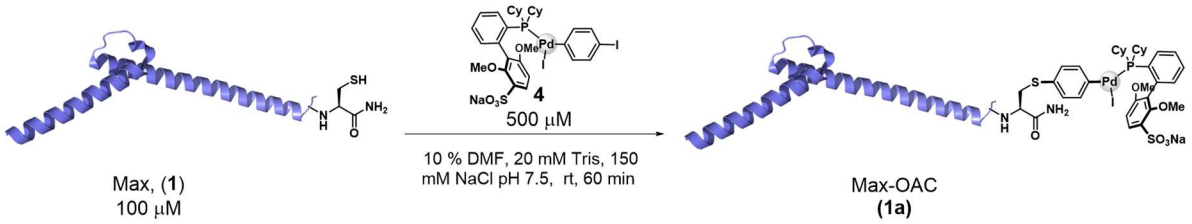


Figure S14. SDS-PAGE analysis of the crude cross-coupling reactions (homodimers). SDS-PAGE analysis of the crude conjugation reaction of the homodimer analogs. Analysis carried out as described in section 1.3. Note: for the analysis of the crude conjugation reactions, samples were incubated at 95 °C for 5 min prior loading into the gel. General note for the conjugation reactions: White precipitations were observed during the reaction. For RP-HPLC purification the crude reaction mixture was diluted by 50% using 50% acetonitrile/water + 0.1% TFA .

7. Synthesis of the protein OACs

7.1 Max OAC



To a Falcon 15mL Conical Centrifuge Tube was added Max **1** (9.0 mL, 1.1 mg/ml, 1.0 equiv) as a solution in 20 mM Tris, 150 mM NaCl (pH 7.5), and Pd OAC **4** (1.0 mL, 4.8 mg/mL, 5.0 equiv) as a solution in DMF. The final reaction concentration of the major reaction components were the following: **1** (100 μM); **4** (500 μM). The Falcon Tube was closed vortexed and incubated at room temperature for 30 min. A small aliquot was taken from the reaction mixture for analysis by LC-MS. Finally, the reaction was purified by RP-HPLC using method B to obtain to obtain the purified Max OAC in 54% isolated yield (5.8 mg).

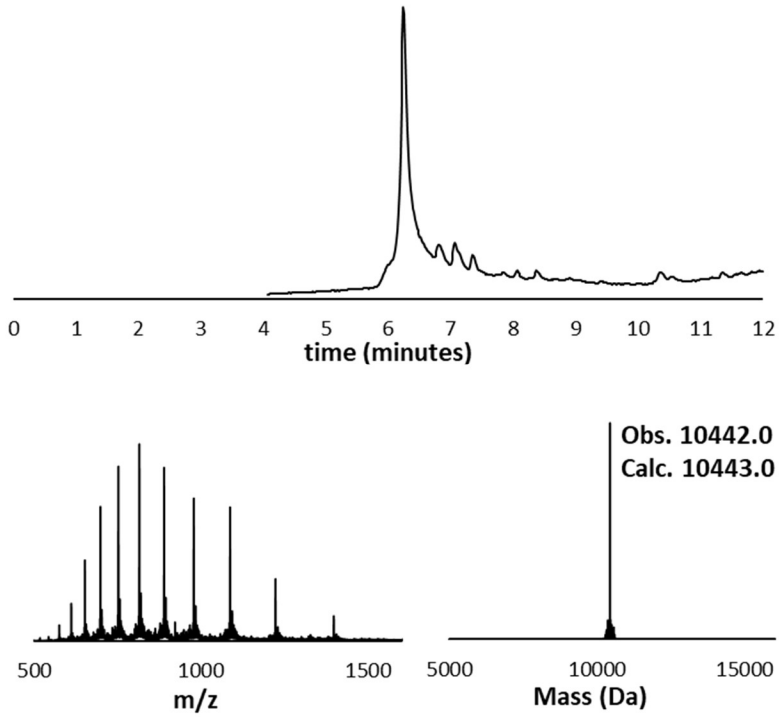
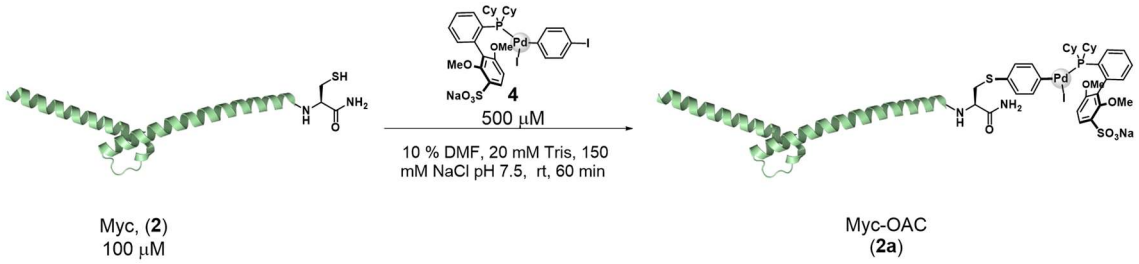


Figure S15. LC-MS analysis of purified Max-OAC (1a). Total ion current chromatogram (TIC), mass-to-charge (m/z) spectrum, and deconvoluted mass spectrum of **1a**. LC-MS analysis was carried out using Method D (see section 1.2).

7.2 Myc OAC



To a Falcon 15mL Conical Centrifuge Tube was added Myc **2** (7.0 mL, 1.1 mg/ml, 1.0 equiv) as a solution in 20 mM Tris, 150 mM NaCl (pH 7.5), and Pd OAC **4** (0.8 mL, 5.4 mg/mL, 5.0 equiv) as a solution in DMF. The final reaction concentration of the major reaction components were the following: **2** (100 μM); **4** (500 μM). The Falcon Tube was closed, vortexed and incubated at room temperature for 30 min. A small aliquot was taken from the reaction mixture for analysis by LC-MS. Finally, the reaction was purified by RP-HPLC using method B to obtain to obtain the purified Myc OAC in 45% isolated yield (3.9 mg).

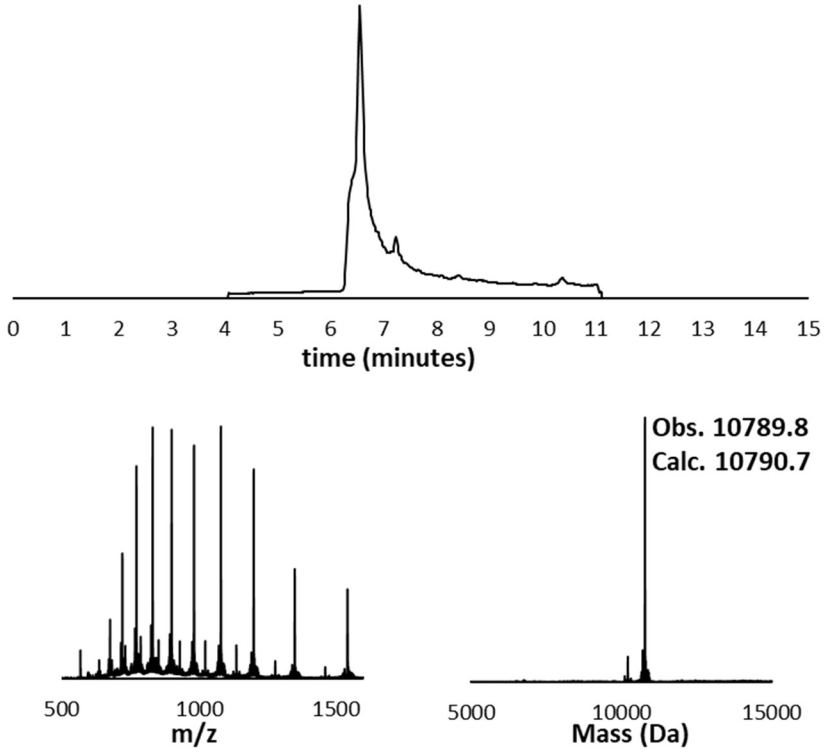
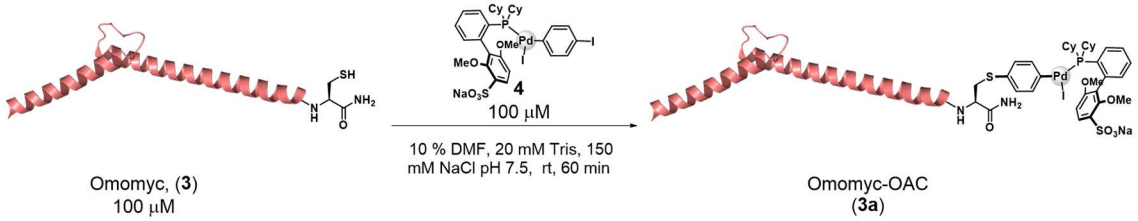


Figure S16. LC-MS analysis of purified Myc-OAC (2a). Total ion current chromatogram (TIC), mass-to-charge (m/z) spectrum, and deconvoluted mass spectrum of **2a**. LC-MS analysis was carried out using Method B (see section 1.2).

7.3 Omomyc OAC



To a Falcon 15mL Conical Centrifuge Tube was added Omomyc **3** (7.0 mL, 1.2 mg/ml, 1.0 equiv) as a solution in 20 mM Tris, 150 mM NaCl (pH 7.5), and Pd OAC **4** (0.7 mL, 5.3 mg/mL, 5.0 equiv) as a solution in DMF. The final reaction concentration of the major reaction components were the following: **3** (100 μM); **4** (500 μM). The Falcon Tube was closed, vortexed and incubated at room temperature for 30 min. A small aliquot was taken from the reaction mixture for analysis by LC-MS. Finally, the reaction was purified by RP-HPLC using method B to obtain to obtain the purified Omomyc OAC in 43% isolated yield (3.9 mg).

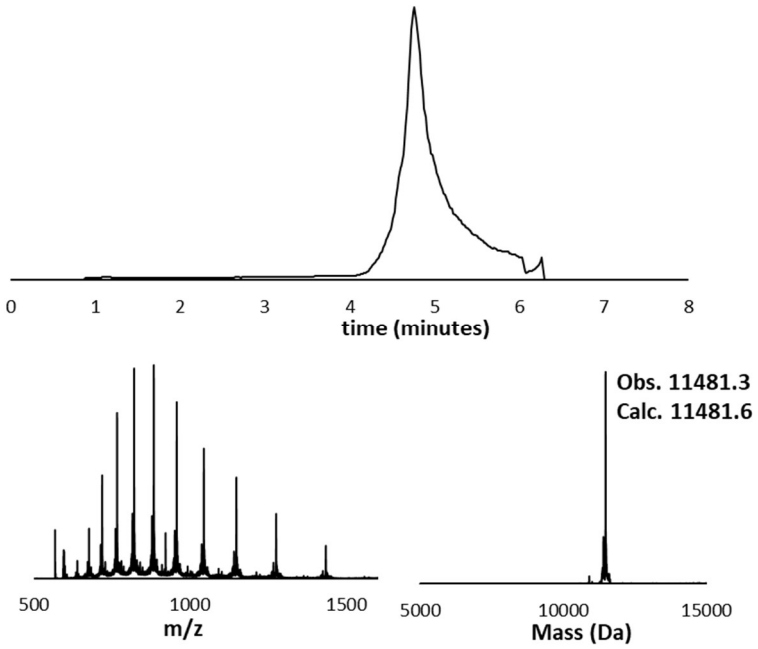
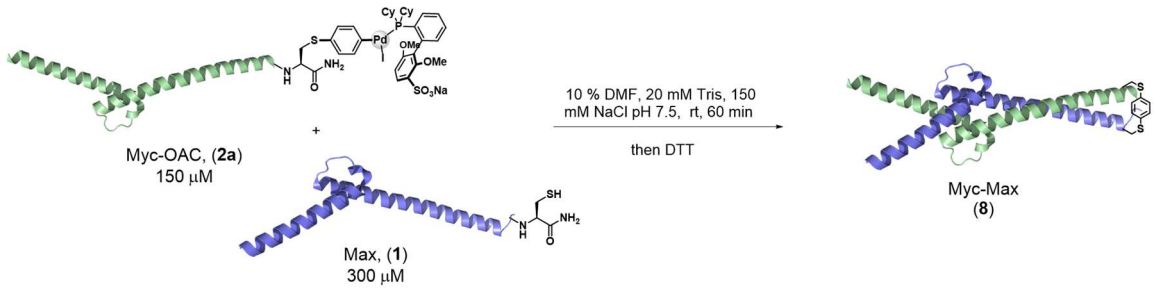


Figure S17. LC-MS analysis of purified Omomyc-OAC (3a). Total ion current chromatogram (TIC), mass-to-charge (m/z) spectrum, and deconvoluted mass spectrum of **3a**. LC-MS analysis was carried out using Method C (see section 1.2).

8. Synthesis of the heterodimer analogs

8.1 Myc-Max



To a 5.0 mL Eppendorf tube was added Myc OAC **2a** (500 µL, 6.0 mg/mL, 1.0 equiv) as a solution in 20 mM Tris, 150 mM NaCl (pH 7.5), 260 µl 20 mM Tris, 150 mM NaCl buffer (pH 7.5), 185 µl DMF, and Max **1** (905 µl, 6.0 mg/ml, 2.0 equiv) as a solution in 20 mM Tris, 150 mM NaCl buffer (pH 7.5). The final reaction concentration of the major reaction components were the following: **2a** (150 µM); **1** (300 µM). The Eppendorf tube was closed, vortexed and incubated at room temperature for 60 min. A small aliquot was taken from the reaction mixture for analysis by SDS-PAGE (Figure S21). Finally, the reaction was quenched by DTT (10 µl, 1 M in H_2O) and kept at room temperature for 5 min, then purified by RP-HPLC using method A to obtain the purified Myc-Max in 7% isolated yield.

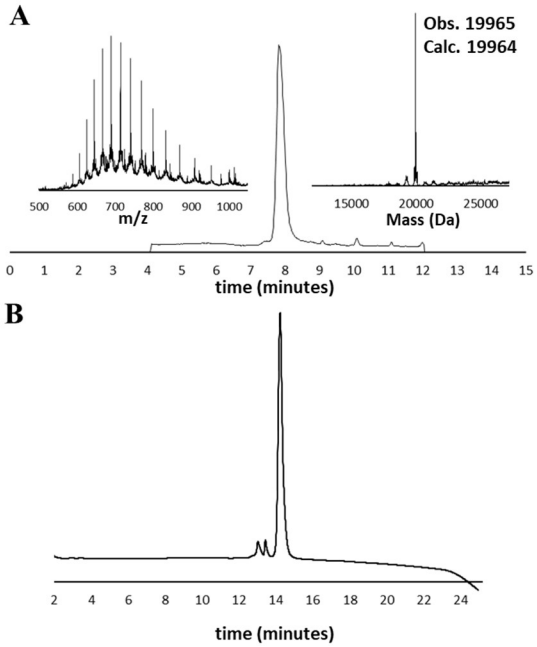
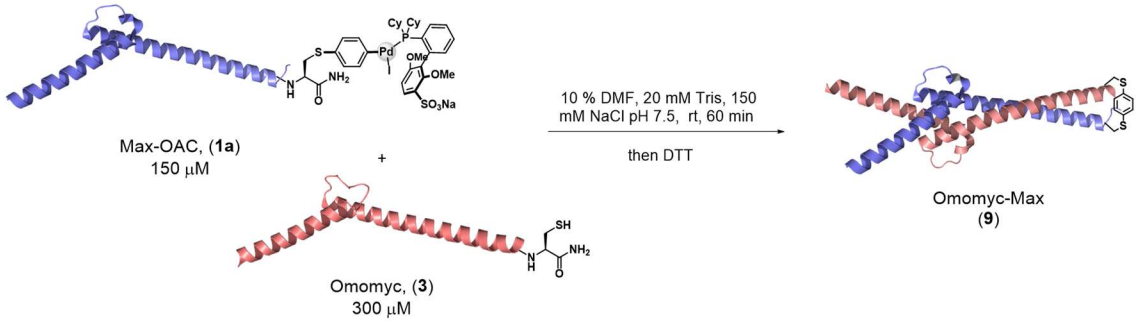


Figure S18. LC-MS and RP-HPLC analysis of purified Myc-Max (8). A) Total ion current chromatogram (TIC), mass-to-charge (m/z) spectrum, and deconvoluted mass spectrum of **8**. LC-MS analysis was carried out using Method E (see section 1.2). B) HPLC chromatogram of the UV absorbance at 214 nm. HPLC analysis was carried out with Method A (see section 1.3).

8.2 Omomyc-Max



To a 5.0 mL Eppendorf tube was added Max OAC **1a** (500 µL, 6.0 mg/mL, 1.0 equiv) as a solution in 20 mM Tris, 150 mM NaCl (pH 7.5), 190 µl 20 mM Tris, 150 mM NaCl buffer (pH 7.5), 190 µl DMF, and Omomyc **3** (1035 µl, 6.0 mg/ml, 2.0 equiv) as a solution in 20 mM Tris, 150 mM NaCl buffer (pH 7.5). The final reaction concentration of the major reaction components were the following: **1a** (150 µM); **3** (300 µM). The Eppendorf tube was closed vortexed, and incubated at room temperature for 60 min. A small aliquot was taken from the reaction mixture for analysis by SDS-PAGE (Figure S21). Finally, the reaction was quenched by DTT (10 µl, 1 M in H_2O) and kept at room temperature for 5 min, then purified by RP-HPLC using method A to obtain the purified Omomyc-Max in 16% isolated yield.

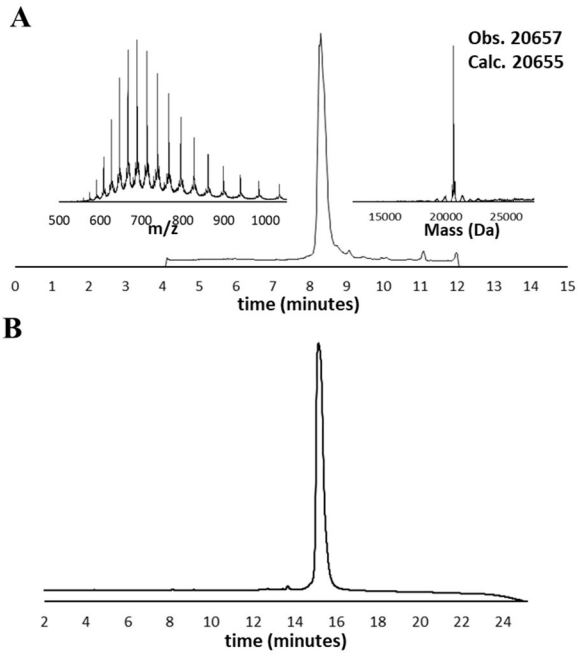
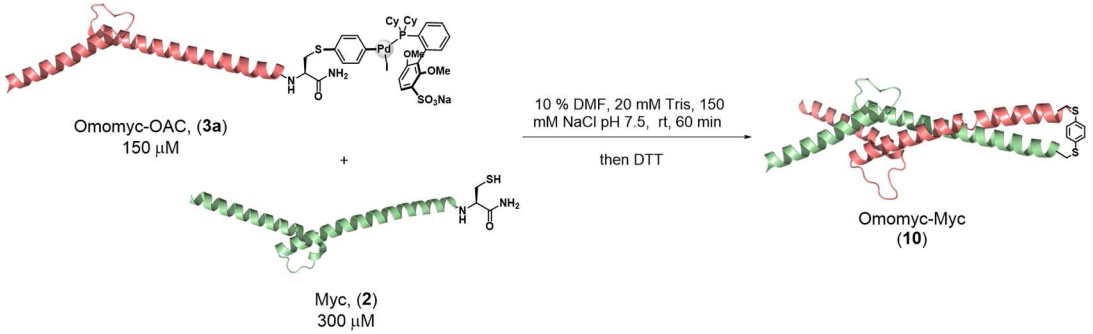


Figure S19. LC-MS and RP-HPLC analysis of purified Omomyc-Max (9). A) Total ion current chromatogram (TIC), mass-to-charge (m/z) spectrum, and deconvoluted mass spectrum of **9**. LC-MS analysis was carried out using Method E (see section 1.2). B) HPLC chromatogram of the UV absorbance at 214 nm. HPLC analysis was carried out with Method A (see section 1.3).

8.3 Omomyc-Myc



To a 5.0 mL Eppendorf tube was added Omomyc OAC **3a** (500 µL, 6.0 mg/mL, 1.0 equiv) as a solution in 20 mM Tris, 150 mM NaCl (pH 7.5), 187 µl 20 mM Tris, 150 mM NaCl buffer (pH 7.5), 174 µl DMF, and Myc **2** (881 µl, 6.0 mg/ml, 2.0 equiv) as a solution in 20 mM Tris, 150 mM NaCl buffer (pH 7.5). The final reaction concentration of the major reaction components were the following: **3a** (150 µM); **2** (300 µM). The Eppendorf tube was closed, vortexed and incubated at room temperature for 60 min. A small aliquot was taken from the reaction mixture for analysis by SDS-PAGE (Figure S21). Finally, the reaction was quenched by DTT (10 µl, 1 M in H_2O) and kept at room temperature for 5 min, then purified by RP-HPLC using method A to obtain the purified Omomyc-Myc in 6% isolated yield.

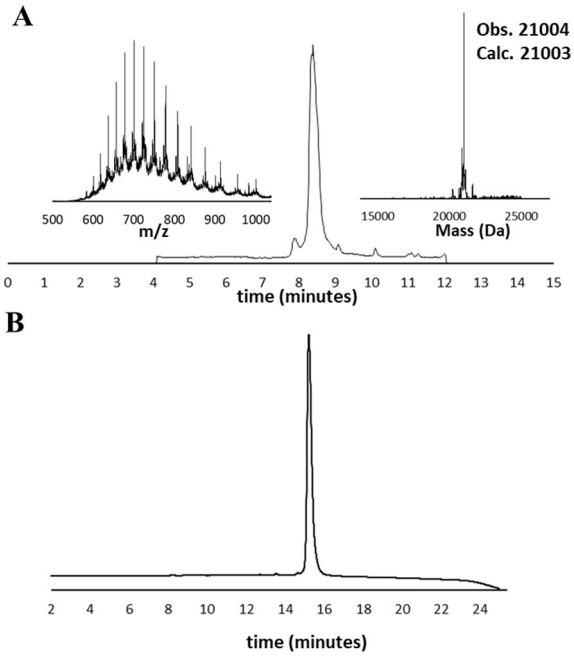


Figure S20. LC-MS and RP-HPLC analysis of purified Omomyc-Myc (10). A) Total ion current chromatogram (TIC), mass-to-charge (m/z) spectrum, and deconvoluted mass spectrum of **10**. LC-MS analysis was carried out using Method E (see section 1.2). B) HPLC chromatogram of the UV absorbance at 214 nm. HPLC analysis was carried out with Method A (see section 1.3).

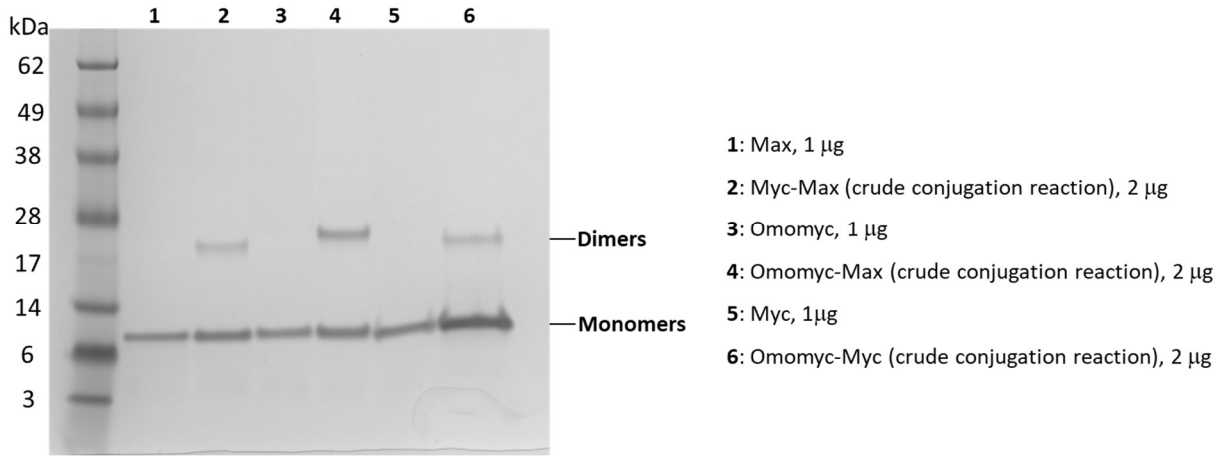


Figure S21. SDS-PAGE analysis of the crude cross-coupling reactions (heterodimers). SDS-PAGE analysis of the crude conjugation reaction of the heterodimer analogs. Analysis carried out as described in section 1.3. Note: for the analysis of the crude conjugation reactions, samples were incubated at 95 °C for 5 min prior loading into the gel

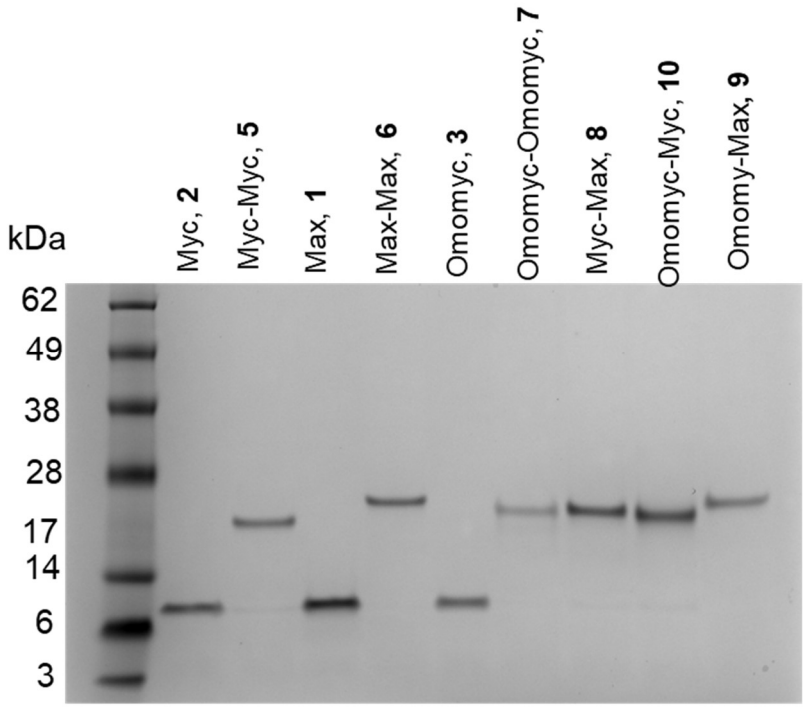
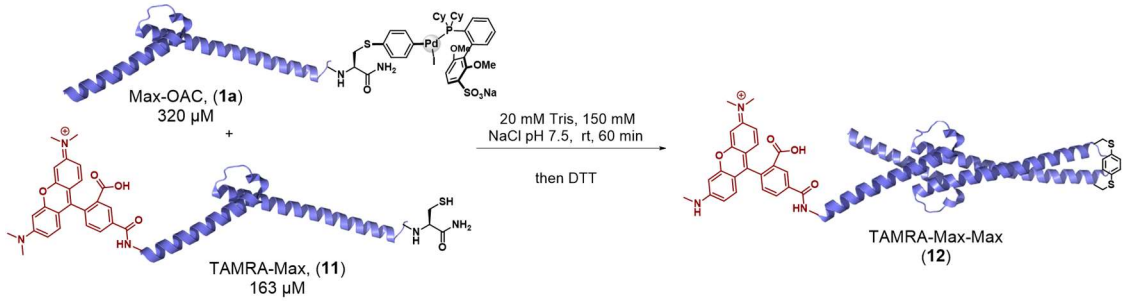


Figure S22. SDS-PAGE analysis of the purified protein monomers and dimers. SDS-PAGE analysis carried out as described in section 1.3.

8.4 TAMRA-Max-Max (12):



To a 1.5 mL Eppendorf tube was added TAMRA-Max **11** (400 µL, 5.0 mg/mL, 1.0 equiv) as a solution in 20 mM Tris, 150 mM NaCl (pH 7.5), and Max OAC **1a** (800 µl, 5.0 mg/ml, 2.0 equiv) as a solution in 20 mM Tris, 150 mM NaCl buffer (pH 7.5). The final reaction concentration of the major reaction components were the following: **11** (163 µM); **1a** (320 µM). The Eppendorf tube was closed, vortexed and incubated at room temperature for 60 min. A small aliquot was taken from the reaction mixture for analysis by LC-MS (Figure S23). Finally, the reaction was quenched by DTT (10 µl, 1 M in H_2O) and kept at room temperature for 5 min, then purified by RP-HPLC using method A to obtain the purified TAMRA-Max-Max in 18% isolated yield.

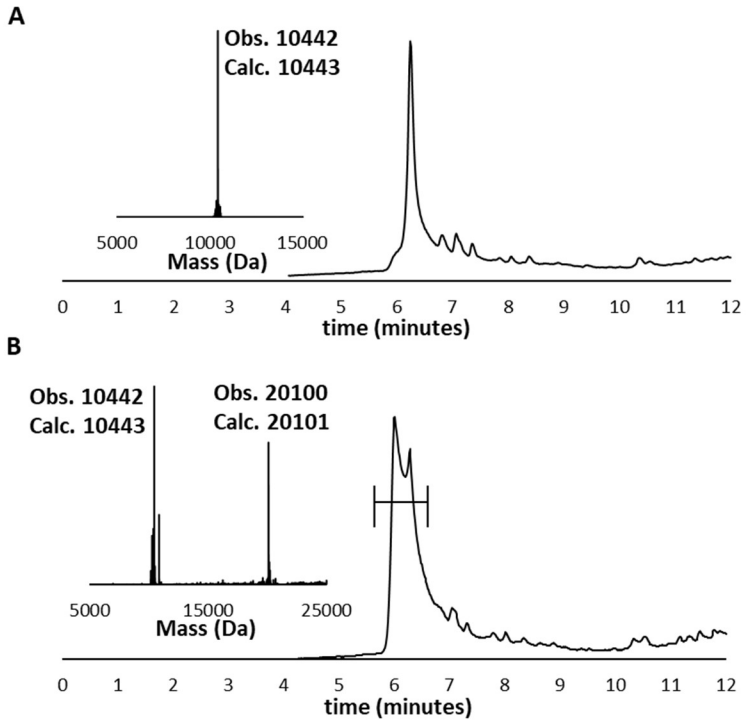


Figure S23. LC-MS analysis of the synthesis of TAMRA-Max-Max (12). Total ion current chromatogram (TIC) and the deconvoluted mass spectrum of the crude reaction. LC-MS analysis was carried out using Method D (see section 1.2). A) Max-OAC **1A** at time zero. B) crude conjugation reaction after 1 h.

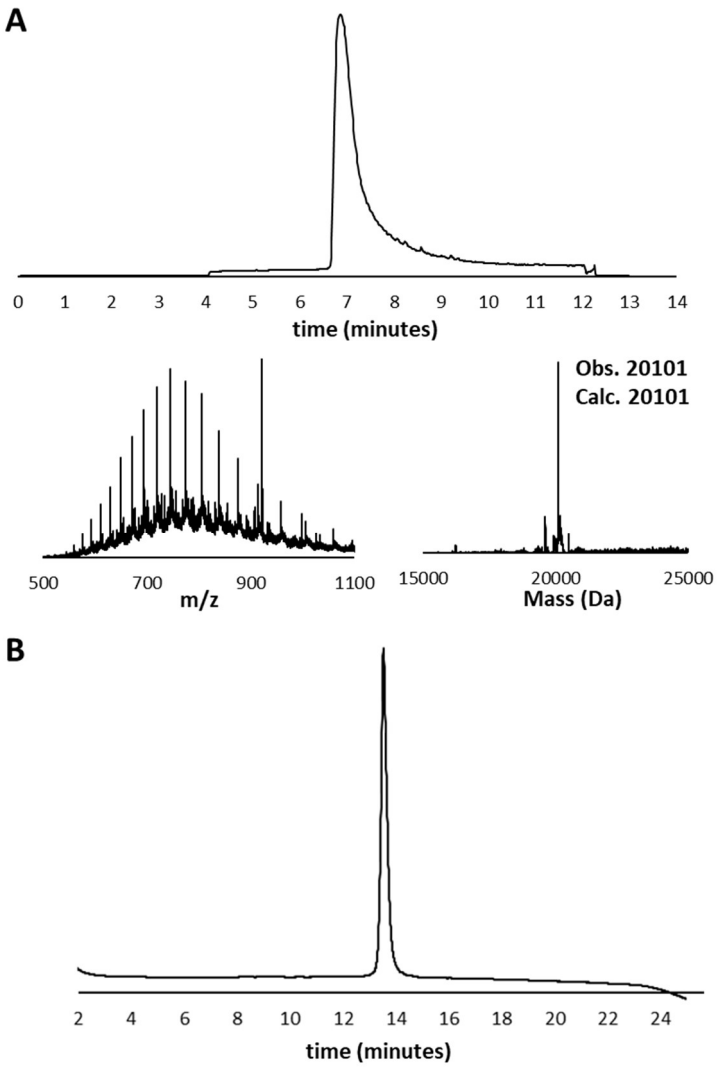


Figure S24. LC-MS and RP-HPLC analysis of purified TAMRA-Max-Max (12). A) Total ion current chromatogram (TIC), mass-to-charge (m/z) spectrum, and deconvoluted mass spectrum of **12**. LC-MS analysis was carried out using Method B (see section 1.2). B) HPLC chromatogram of the UV absorbance at 214 nm. HPLC analysis was carried out with Method A (see section 1.3).

9. Large scale synthesis of Max-Max analog

To a Falcon 15mL Conical Centrifuge Tube was added 10.0 mg of Max **1** (1.9 mL, 1.0 equiv) as a solution in 20 mM Tris, 150 mM NaCl (pH 7.5), Pd OAC **4** (100.0 µL, 10.0 mg/mL, 1.0 equiv) as a solution in DMF (titrated over one minute), and 110.0 µL DMF. The final reaction concentration of the major reaction components were the following: **1** (0.5 mM); **4** (0.5 mM). The Eppendorf tube was closed and mixture up and down, and incubated at room temperature for 60 min. A small aliquot was taken from the reaction mixture for analysis by LC-MS (Figure S25). Finally, the reaction was quenched by DTT (50 µl, 1 M in H_2O) and kept at room temperature for 5 min, then purified by RP-HPLC using method A to obtain the purified Max-Max in 38% isolated yield.

LCMS spectra of Max-Max synthesis
(TIC, m\z & deconvoluted mass)
LCMS method D

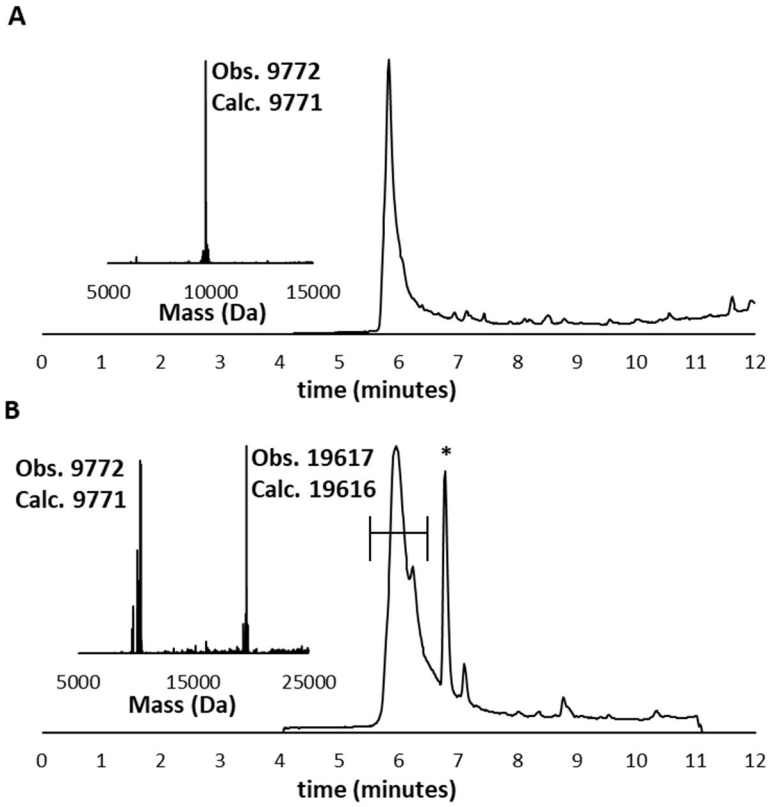


Figure S25. LC-MS analysis of the synthesis of Max-Max 6. Total ion current chromatogram (TIC) and the deconvoluted mass spectrum of the crude reaction. LC-MS analysis was carried out using Method D (see section 1.2). A) Max **1** at time zero, B) Crude conjugation reaction after 1 h. * sSPhos ligand

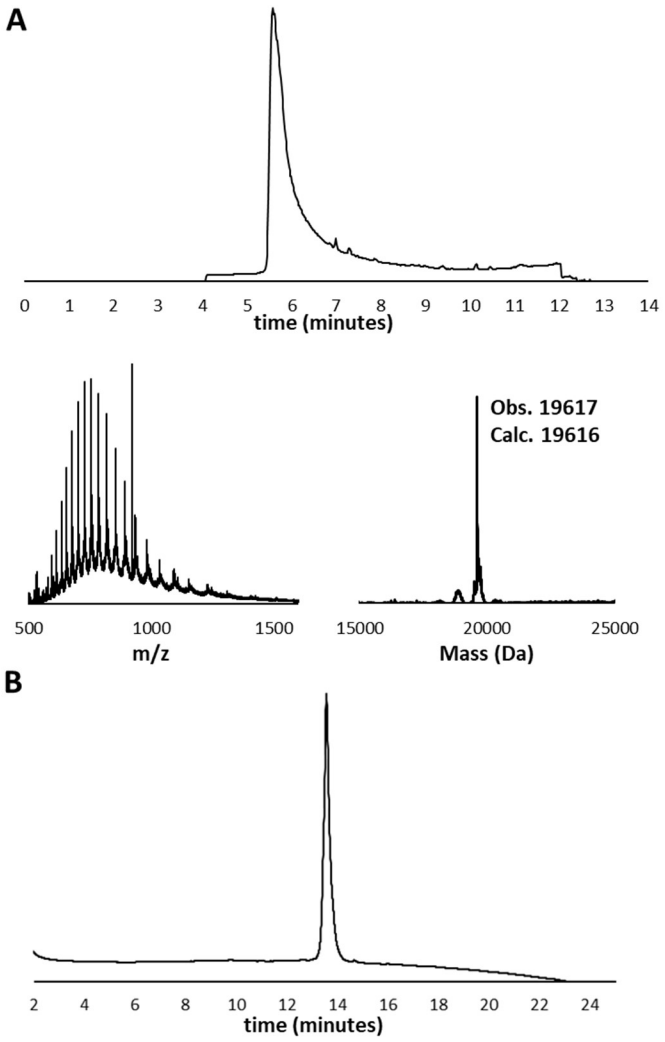


Figure S26. LC-MS and RP-HPLC analysis of purified Max-Max (6). A) Total ion current chromatogram (TIC), mass-to-charge (m/z) spectrum, and deconvoluted mass spectrum of **6**. LC-MS analysis was carried out using Method B (see section 1.2). B) HPLC chromatogram of the UV absorbance at 214 nm. HPLC analysis was carried out with Method A (see section 1.3).

10. DNA-Binding analysis and Electrophoretic Mobility-Shift Assay (EMSA)

Monomers: To 0.6 mL Eppendorf tube was added the E-box DNA probe (5.0 µL, 10.0 µM) as a solution in 10 Mm MES, 150 mM KCl, 1mM $MgCl_2$, 10% glycerol buffer, 41.0 µL 10 Mm MES, 150 mM KCl, 1mM $MgCl_2$, 1 mM TCEP ,10% glycerol buffer, and target protein monomer analog (4.0 µL, 50.0 µM) as a solution in 10 Mm MES, 150 mM KCl, 1mM $MgCl_2$, 1 mM TCEP, 10% glycerol buffer (for homodimers: Max+Max, Myc+Myc, Omomyc+Omomyc) or (2.0 µL, 50.0 µM) of protein monomer as a solution in 10 Mm MES, 150 mM KCl, 1mM $MgCl_2$, 1 mM TCEP, 10% glycerol buffer (for heterodimers: Myc+Max, Omomyc+Max, Omomyc+Myc). The final reaction concentration of the major reaction components was the following: DNA (1.0 µM); protein (2.0 µM). The Eppendorf tube was closed mixture up and down and incubated at room temperature for 1 hour. During the incubation, a 10 % TBE gel, 1.0mm x 10 well, was prerun in 1x TBE buffer (1 h, 4 °C, 75V). Next, the DNA-binding activity of each dimer analog was analyzed by EMAS. 10.0 µL of DNA-protein mixture was mixed with 2.0 µL DNA Loading Dye (6X) loaded to the gel, which was run at 75V at 4 °C for 90 min. The gel was washed two times with water for 30 seconds and then stained via 0.02 % ethidium bromide in 1xTBE buffer for 15 min at room temperature. Bands were visualized on a Biorad Gel imager. *Note: The E-box DNA probe (10 µM) solution in 10 Mm MES, 150 mM KCl, 1mM $MgCl_2$, 10% glycerol buffer, was heated to 95 °C for 5 minutes and then let cool down to room temperature over 10 minutes.*

Dimers: To 0.6 mL Eppendorf tube was added the E-box DNA probe (5.0 µL, 10.0 µM) as a solution in 10 Mm MES, 150 mM KCl, 1mM $MgCl_2$, 1 mM TCEP, 10% glycerol buffer, 43.0 µL 10 Mm MES, 150 mM KCl, 1mM $MgCl_2$, 1 mM TCEP, 10% glycerol buffer, and target protein dimer (2.0 µL, 50.0 µM) as a solution in 10 Mm MES, 150 mM KCl, 1mM $MgCl_2$, 1 mM TCEP, 10% glycerol buffer. The final reaction concentration of the major reaction components was the following: **DNA** (1.0 µM); protein dimer (2.0 µM). The Eppendorf tube was closed mixture up and down and incubated at room temperature for 1 hour. During the incubation, a 10 % TBE gel, 1.0mm x 10 well, was prerun in 1x TBE buffer (1 h, 4 °C, 75V). Next, the DNA-binding activity of each dimer analog was analyzed by EMAS. 20.0 µL of DNA-protein mixture was mixed with 4.0 µL DNA Loading Dye (6X) loaded to the gel, which was run at 75V at 4 °C for 90 min. The gel was washed two times with water for 30 seconds and then stained via 0.02 % ethidium bromide in 1xTBE buffer for 15 min at room temperature. Bands were visualized on a Biorad Gel imager. *Note: The E-Box DNA probe (10 µM) solution in 10 Mm MES, 150 mM KCl, 1mM $MgCl_2$, 10% glycerol buffer, was heated to 95 °C for 5 minutes and then let cool down to room temperature over 10 minutes.*

11. Circular dichroism (CD) analysis

CD analysis was carried out using AVIV 420 circular dichroism spectrometer with a 1 mm path length quartz cuvette. 300.0 µL protein was prepared in 0.1 mg/ml in MES 10 mM, KCl 150 mM, 1 mM $MgCl_2$, 1 mM TCEP, glycerol 10% buffer (pH 6.5). CD spectra of all samples were recorded at 25 °C from 250 nm to 190 nm with three seconds averaging times at each wavelength.

12. Melting point study

Melting point analysis was carried out using AVIV 420 circular dichroism spectrometer with a 1 mm path length quartz cuvette. 300.0 µL protein was prepared in 0.1 mg/ml in MES 10 mM, KCl 150 mM, 1 mM $MgCl_2$, 1 mM TCEP, glycerol 10% buffer (pH 6.5). CD signals at 222 nM were recorded at temperature points between 4 to 89 °C with 5 °C intervals and equilibration times of 1 min at each temperature.

13. Octet BioLayer Interferometry binding assay

Biolayer interferometry (BLI) assays were performed using an Octet Red96 System (ForteBio; Menlo Park, CA) in 96 well plates (GreinerBio-One; Kremsmünster, Austria; polypropylene, flat-bottom, chimney well). Streptavidin Octet biosensors (ForteBio; Menlo Park, CA) were dipped into 200 µL 1 µM of biotinylated E-box DNA probe in 0.1% BSA, 0.02% Tween-20, 1x PBS (kinetic buffer) for the loading step (185 sec). Sensors were then dipped into kinetic buffer for 60 sec. Next, the tips loaded with Max-Max **6** prepared in kinetic buffer at indicated concentrations for 800 sec to obtain the association curve. Finally, the tips were dipped into kinetic buffer for 3000 sec to obtain the dissociation curve. Measurements were carried out at 30 °C. Data were analyzed within the ForteBio Data Analysis software. The association and dissociation curves are fitted with Fortebio Biosystems (global fitting algorism) to obtain the K_D. Kinetic K_D is reported.

14. Cell culture

HeLa (ATCC CCL-2), A549 (ATCC CCL-185), and H441 (ATCC HTB-174) cancer cell lines were maintained in MEM, FK-12, and RPMI-1640 media each containing 10% v/v fetal bovine serum (FBS) and 1% v/v penicillin-streptomycin, respectively, at 37 °C and 5% CO_2. Cells were passaged at 80% confluency using 0.25% trypsin-EDTA.

15. Flow cytometry

HeLa cells were plated at 10,000 cells/well in a 96-well plate the night before the experiment. On the day of, cells were treated with the indicated concentrations of TAMRA-Max-Max (**12)** for 15 minutes in serum-containing culture medium, washed once with PBS, and treated with 0.25% trypsin-EDTA for 30 minutes to digest membrane-bound protein. Cells were then washed with PBS, incubated in PBS containing 1x DAPI for three minutes, and then resuspended in PBS containing 2% FBS. Cells were then immediately analyzed on a BD FACS LSR II using DAPI and PE channels.

Gating was performed to include cells occupying the main population of healthy cells; gates were generated for the untreated control and then were applied to the experimental samples. Shown below are examples of the gating strategy, generated for untreated cells (top) and applied to an experimental sample (bottom), which is the highest concentration of TAMRA-Max-Max **12** tested. Because there is no population of DAPI-positive cells, it is clear that the cells did not suffer from membrane toxicity after treatment with the conjugate.

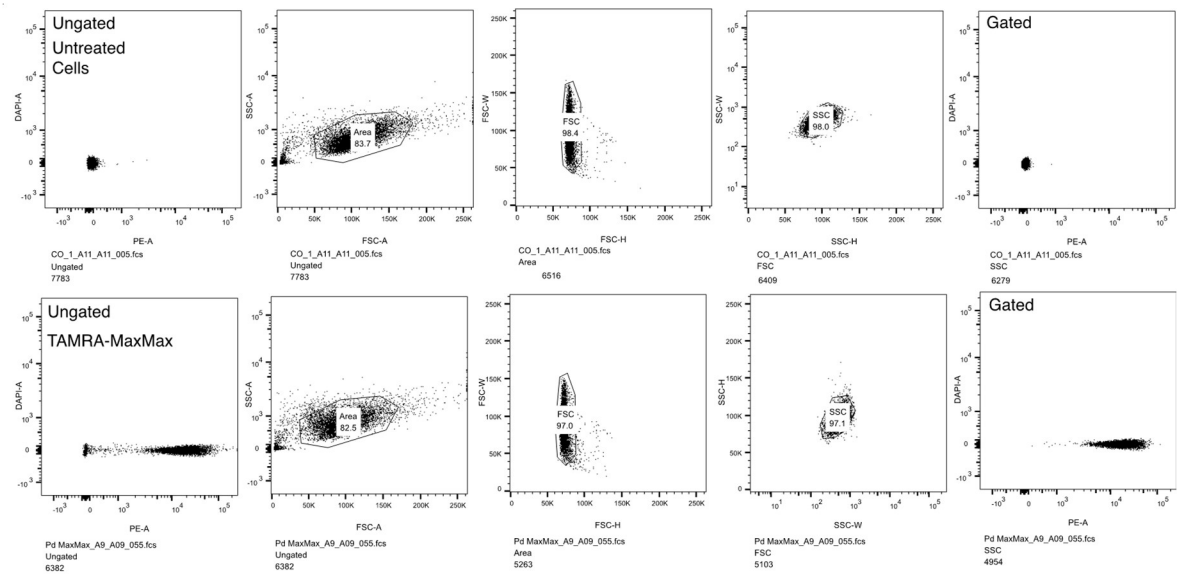


Figure S27. Flow cytometry gating. Histograms of the experiments in which the highest concentration of TAMRA-Max-Max **12** used, before and after gating. Because there is no population of DAPI-positive cells, it is clear that the cells did not suffer from membrane toxicity after treatment with the conjugate **12**.

16. Cell proliferation assay

Cells were plated at 5,000 cells/well in a 96-well plate the day before the experiment. Max-Max **6** was prepared at varying concentrations in complete media and transferred to the plate. Cells were incubated at 37 °C and 5% CO_2 for 72 h, and cell proliferation was measured using the CellTiter-Glo assay quantified by luminescence. Experimnets were performed in triplicates.

17. Microscopy

HeLa cells were plated at 10,000 cells/well in a 96-well 30mm glass-bottom plate the night before the experiment. On the day of, cells were treated with TAMRA-Max-Max **12** (5 μM) in complete medium for 15 minutes, washed twice with fresh medium, and incubated at 37 °C and 5% CO_2 for 1 h before imaging. Micrographs were obtained in the W.M. Keck microscopy facility on an RPI Spinning Disk Confocal microscope on RFP setting (561 nm 100mW OPSL excitation laser, 605/70 nm emission) and DAPI setting (405 nm 100mW OPSL excitation laser, 450/50 nm emission).

18. Stability of Max-Max

To 1.5 mL Eppendorf tube was added Max-Max **6** (50 ul, 25 μM) as a solution in 1x PBS (pH 7.5) and incubated at 37 °C for 24 h. The sample was analyzed by LCMS at time zero and after 24 h.

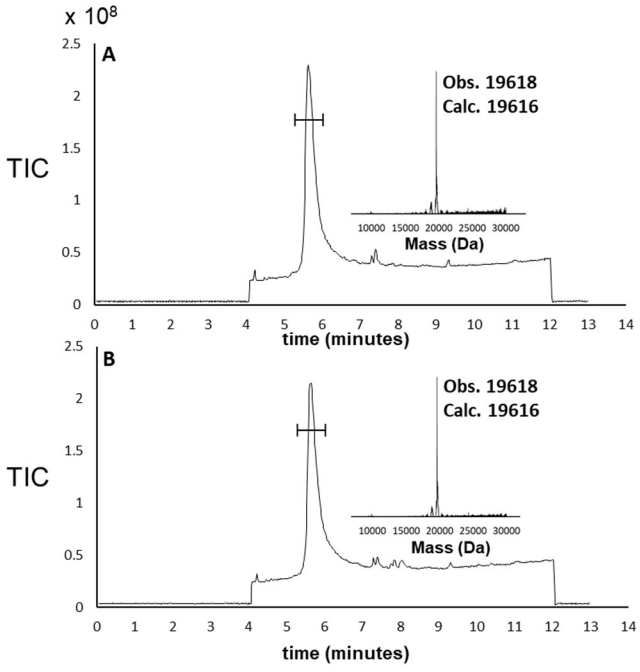


Figure S28. LC-MS analysis for the stability of Max-Max (6). Total ion current chromatogram (TIC) and deconvoluted mass spectrum of **6** before and after incubation for 24 h. LC-MS analysis was carried out using Method B (see section 1.2). A) **6** at time zero. B) **6** after 24 h incubation at 37 °C.

19. Proteolytic stability of Max-Max

Max-Max **6** or Max **1** (12.5 µM) was incubated in normal human serum (NHS) (5% in 1x PBS) for 1 h at 37 °C. The sample was analyzed by LCMS (method D).

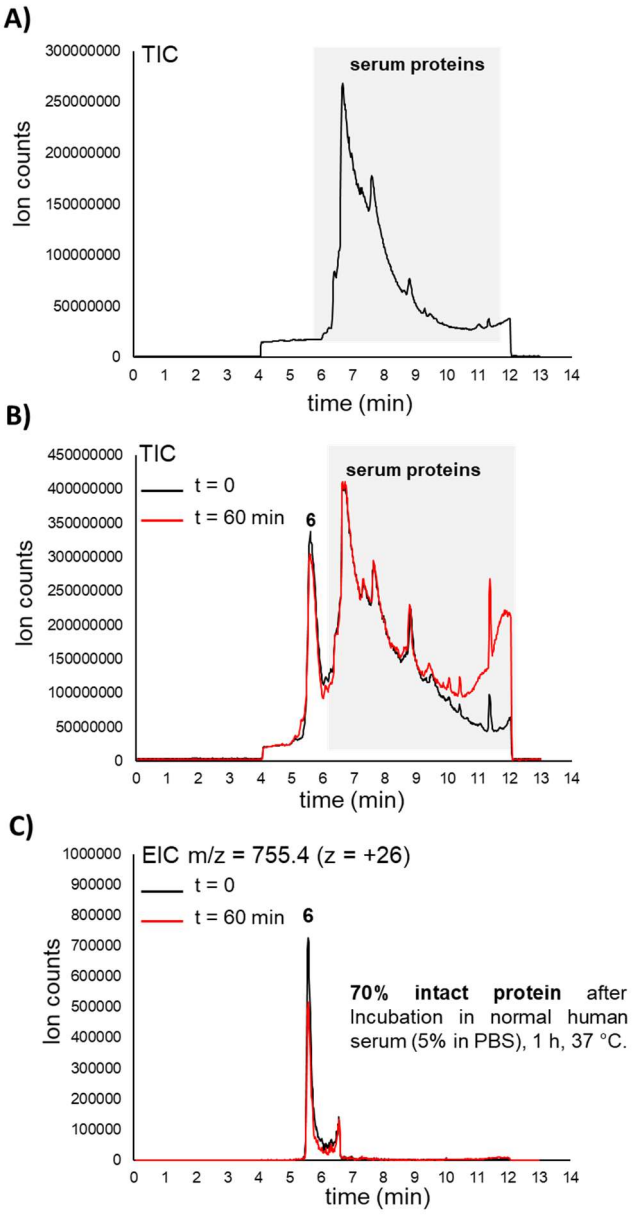


Figure S29. LC-MS analysis of the proteolytic stability of Max-Max (6). Total ion current chromatogram (TIC) and extracted ion current chromatogram (EIC) spectrum of **6** before and after incubation in normal human serum (NHS). LC-MS analysis was carried out using Method B (see section 1.2). A) LC-MS trace (TIC) of human serum proteins. B) LC-MS trace (TIC) of **6** before and after incubation in NHS for 1 h at t = 0 (black) and t = 1 h (red). C) LC-MS trace (EIC) of chromatogram B at t = 0 (black) and t = 1 h (red).

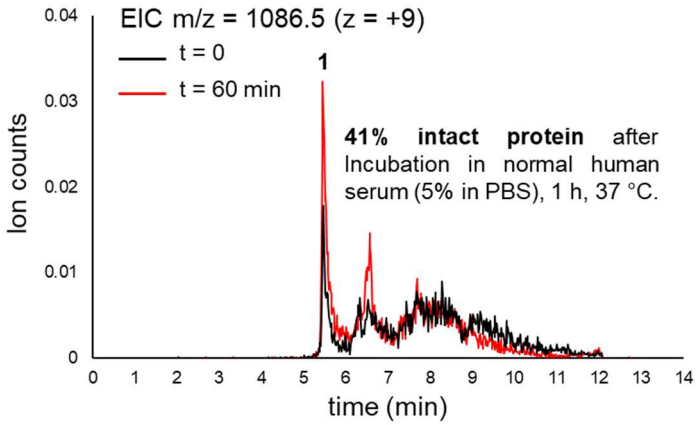


Figure S30. LC-MS analysis of the proteolytic stability of Max (1). Extracted ion current chromatogram (EIC) spectrum of **1** before and after incubation in NHS for 1 h at t = 0 (black) and t = 1 h (red). LC-MS analysis was carried out using Method B (see section 1.2).

20. RNA-sequencing and GSEA

In a 6 well plate, 125,000 A549 cells were plated into each well. The following day, the cells were treated with Max-Max **6** or Omomyc **3** (12.5 μM) in F12K media supplemented with 10% FBS and 1% pen/strep for 72 h. RNA was isolated using the Qiagen RNeasy Plus Mini Kit (74136) followed by DNAse treatment (AM1906). The experiment was performed in duplicates. KAPAHyperRiboErase libraries were prepared and sequenced on a Hi-seq 2500 instrument. Reads from sequencing were aligned using HISAT2 htseq-count function. Differential gene expression analysis between treated and control cells was performed using DESEQ2 package in R on raw aligned read counts. The differentially expressed genes were ranked by their log2FC and adjusted p-value. Pre-ranked Gene Set Enrichment Analysis (GSEA) was performed using gene sets in Molecular Signatures Database (MSigBD) to identify enriched MYC-target gene sets.

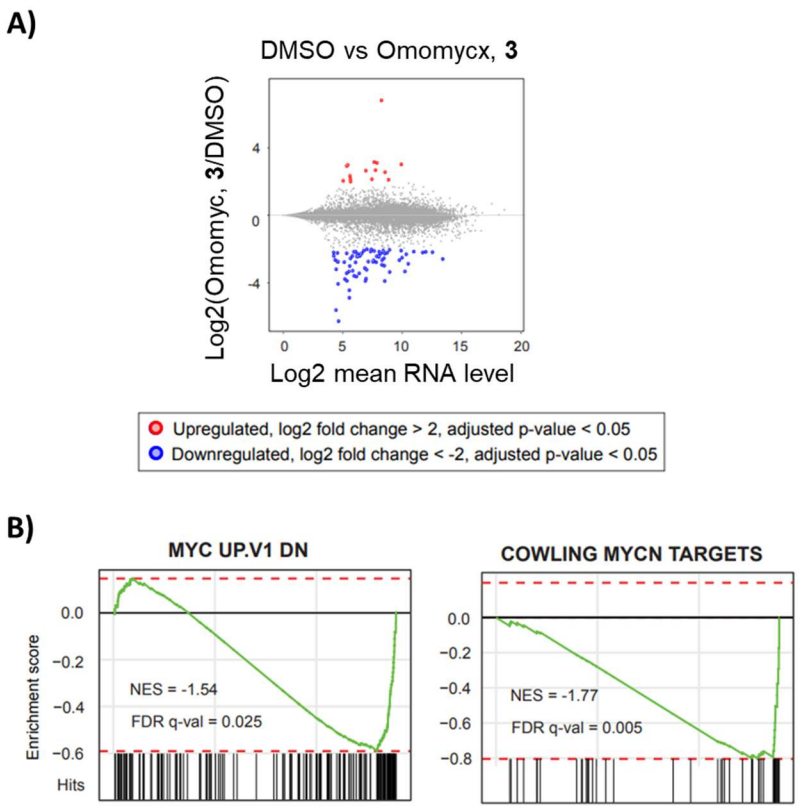


Figure S31. RNA-seq and GSEA analysis for A549 cells treated with Omomyc 3. A) Shown is a MA plot of differentially expressed genes in A549 cells treated with Omomyc **3** compared to DMSO. RNA-Sequencing analysis: A549 cells were treated with Omomyc **6** (12.5 mM) or DMSO as a control for 72 h, followed by RNA extraction and sequencing. Gray dots represent unaffected genes (cells treated with DMSO); blue dots represent downregulated genes; red dots represent upregulated genes (treated cells with **3**. Upregulated genes with adjusted p-value < 0.05 and |log2FC| ³ 1 shown in red, downregulated genes with p-value < 0.05 and |log2FC| £ 1 are shown in blue. The experiment was performed in duplicates. B) Gene set enrichment analysis (GSEA) comparing gene expression of treated cells with Omomyc **3** vs. DMSO. Enrichment plot of Myc target gene signature show a negative enrichment in Omomyc **3** condition of two Myc-target gene sets (q-value < 0.025 and q-value < 0.005).

21. Inductively coupled plasma mass spectrometry (ICP-MS)

ICP-MS analysis were carried out using an Agilent 7900 ICP-MS system at the MIT Center for Environmental Health Sciences (MIT CEHS). Samples were digested with concentrated nitric acid (67-70%, VWR Aristar® Plus Nitric Acid, product no. 87003-259) for 16 hours at 60 ºC, cooled to room temperature, then diluted with Milli-Q® filtered water to reach a final nitric acid concentration of 5%. Data analyses were carried out using Agilent ICP-MS MassHunter Software. Raw count data were converted to concentrations utilizing a standard calibration curve. Calibration standards were prepared for ICP-MS analysis using an analytical standard obtained from Agilent Technologies (Part No. 5190-8497).

To a 5.0 mL Eppendorf tube was added purified Max-Max 6 (500 μL, 0.4 mg/mL) as a solution in 20 mM Tris, 150 mM NaCl buffer (pH 7.5) and 132 μL of concentrated nitric acid for digestion and incubated at 60 ºC for 3 h. Next, the solution mixture was cooled to room temperature and diluted to a volume of 2.0 mL with Milli-Q® filtered water. ICP-MS analysis of this mixture gave a palladium content of 4.7 ppb. Therefore, the original solution had a palladium content of 18.8 ppb (4 * 4.7 ppb). Palladium content relative to protein content: Palladium concentration in protein = 18.8 ppb * (500 mg) / (0.5 mL * 0.4 mg/mL) = 47 ppm. Palladium removed: 99.5 %.

22. Pd-cell viability assay:

25,000 cells were plated per well in a 48-well plate the morning of the experiment. 8 hours later, cells were treated with 0, 1 nM, 100 nM, or 1 μM of Pd in cell culture media supplemented with 10% FBS and 1% v/v penicillin-streptomycin, and incubated at 37 °C and 5% CO_2 for 72 hours. Following incubation, the ATP concentration in each well was measured by CellTiter-Glo and a luminescence counter. There is no statistical difference between the treated and untreated wells, meaning that the Pd is not the cause of toxicity when the Pd cross-coupled protein dimers are used to treat the cells.

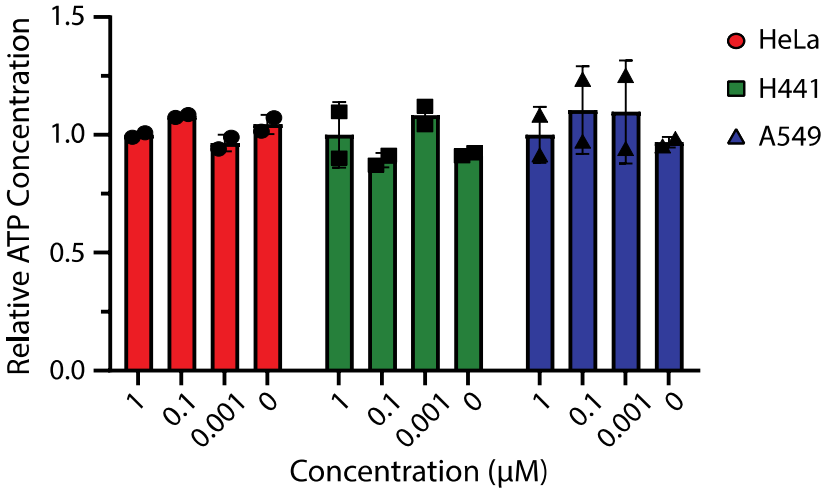


Figure S32. Palladium is nontoxic to cells at tested concentrations. Shown are bar graphs displaying relative ATP concentrations of cells treated with increasing concentrations of Pd, normalized to untreated conditions, as tested by CellTiter-Glo. 1 μM = 500 ppm Pd relative to protein dimer **6**, 0.1 μM = 50 ppm Pd relative to protein dimer **6**, 0.001 μM = 0.5 ppm Pd relative to protein dimer **6**. Pd source: Pd OAC **4** quenched with DTT (to emulate the conditions of the protein conjugation reactions).

22. References

1. Dhanjee, H. H.; Saebi, A.; Buslov, I.; Loftis, A. R.; Buchwald, S. L.; Pentelute, B. L. Protein–Protein Cross-Coupling via Palladium–Protein Oxidative Addition Complexes from Cysteine Residues. *J. Am. Chem. Soc.* **2020**, *142*, 9124.

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