

Synthesis of Biotinylated Episilvestrol: Highly Selective Targeting of the Translation Factors eIF4AI/II

Jennifer M. Chambers,^{1,#} Lisa M. Lindqvist,^{2,#,*} Andrew Webb,² David C. S. Huang,² G. Paul Savage,³ and Mark A. Rizzacasa^{1,*}

masr@unimelb.edu.au; lindqvist@wehi.edu.au

¹School of Chemistry, The Bio21 Institute, The University of Melbourne, Melbourne, Victoria 3010, Australia.

²Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria 3052, Australia and Department of Medical Biology, The University of Melbourne, Parkville, Victoria 3010, Australia.

³CSIRO Molecular and Health Technologies, Bayview Avenue, Victoria 3168, Australia.

These authors contributed equally to this work.

Supporting Information

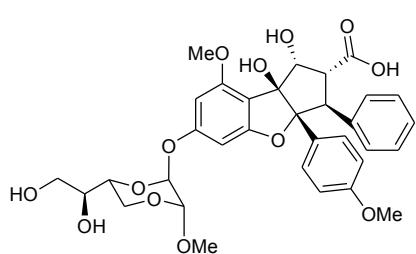
Experimental Section

General	S3
Preparation of episilvestric acid 5 and silvestric acid	S3
Preparation of methyl amide 6	S4
Preparation of propargyl amide 7	S4
Preparation of azide biotin linker 8	S5
Preparation of biotin episilvestrol 9	S6
In vitro translations	S7
Cell viability assay	S7
Rate of translation in cell culture (MEFs)	S7

Streptavidin pulldowns	S8
Figure 1: Specificity of indicated antibodies.	S9
Protein Mass spectroscopy	S9
Figure 2: Analysis of the pulldown assay by mass spectrometry.	S11
Figure 3: eIF4A isoform comparison by mass spectrometry.	S13
References	S15
¹ H NMR Spectrum of episilvestric acid 5	S16
¹³ C NMR Spectrum of epilvestric acid 5	S17
¹ H NMR Spectrum of methyl amide 6	S18
¹³ C NMR Spectrum of methyl amide 6	S19
¹ H NMR Spectrum of propargyl amide 7	S20
¹³ C NMR Spectrum of propargyl amide 7	S21
¹ H NMR Spectrum of azide biotin linker 8	S22
¹³ C NMR Spectrum of azide biotin linker 8	S23
¹ H NMR Spectrum of biotin episilvestrol 9	S24
¹³ C NMR Spectrum of biotin episilvestrol 9	S25

General

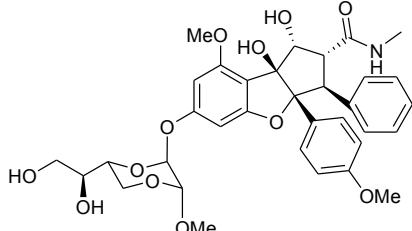
Proton nuclear magnetic resonance spectra (^1H NMR, 500 MHz and 600 MHz) and proton decoupled carbon nuclear magnetic resonance spectra (^{13}C NMR, 125 MHz and 150 MHz) were obtained in deuteriochloroform with residual chloroform as internal standard unless otherwise noted. Chemical shifts are followed by multiplicity, coupling constant(s) (J , Hz), integration, and assignments where possible. Optical rotations were recorded for a 1 mL solution and units are deg. cm^2g^{-1} . Flash chromatography was carried out on silica gel 60. Analytical thin layer chromatography (TLC) was conducted on aluminium-backed 2 mm thick silica gel 60 GF₂₅₄ and chromatograms were visualized with 20% w/w phosphomolybdic acid in ethanol. High resolution mass spectra (HRMS) were obtained by ionizing samples via electron spray ionization (ESI). Anhydrous THF and CH₂Cl₂ were used from a solvent cartridge system. Dry methanol was distilled from magnesium methoxide. All other solvents were purified by standard methods. Petrol used refers to petroleum ether 40-60°C boiling range. All other commercially available reagents were used as received. The standard workup refers to extraction with a particular solvent (3x), washing with water and brine, drying with MgSO₄, and concentration under reduced pressure.



Episilvestric acid 5. To episilvestrol **2** (28.2 mg, 0.431 mmol) in was added 15 ml of 0.4% KOH in 1:2 MeOH: H₂O. The reaction was allowed to stir at 50 °C for 2.5 hr. The reaction was then cooled to 0 °C and diethyl ether and sat. NaCl was added. 1M HCl was added until pH ~2 and the reaction was allowed to warm to rt and stir for 30 min.

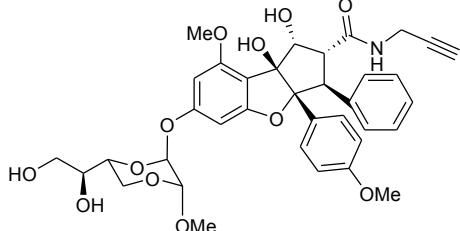
The reaction was extracted 4x with diethyl ether, dried over Na₂SO₄, filtered, and solvent was evaporated. Product as a white solid (27.5 mg, quantitative yield) was used without further purification: $[\alpha]_D^{23} -109.9$ (*c* 0.1520, CH₂Cl₂); IR ν_{max} (film): 3407, 2917, 2849, 1712, 1603, 1514, 1498, 1454, 1430, 1378, 1299, 1251, 1218, 1169, 1132, 1118, 1060, 1029, 964, 901, 884, 826, 770, 733, 699 cm⁻¹; ^1H NMR (600 MHz) δ 7.08-7.05 (m, 5H), 6.90 (d, J = 5.4 Hz, 2H), 6.65 (d, J = 8.7 Hz, 2H), 6.41 (brs, 1H), 6.26 (brs, 1H), 5.31 (s, 1H), 5.03 (brs, 1H), 4.60 (s, 1H), 4.29 (d, J = 10.6 Hz, 1H), 4.08 (brs, 1H), 3.97 (t, J = 10.9 Hz, 1H), 3.88-3.78 (m, 2H), 3.84 (s, 3H), 3.70 (s, 3H), 3.65-3.54 (m, 3H), 3.51 (s, 3H); ^{13}C NMR (150MHz) δ 173.5, 160.7, 159.9, 158.9, 157.4, 136.9, 129.0, 128.1, 128.0, 126.8, 126.5, 112.9, 108.7, 102.0, 95.4, 94.9, 93.6, 93.3, 92.6, 79.8, 76.7, 60.1, 56.1, 55.29, 55.25, 55.21, 50.2; HRMS

(ESI): Calculated for $C_{33}H_{36}NaO_{13} [M+Na]^+$ 663.2048, found 663.2047. Silvestric acid was made in the same manner and spectroscopically identical to previously reported compound.³



Methyl amide 6. To a solution of episilvestric acid **5** (3.4 mg, 0.005 mmol) in CH_2Cl_2 (1.5 mL) at 0 °C was added DCC (7.4 mg, 0.036 mmol) and methyl amine hydrochloride (3.1 mg, 0.046 mmol), the resulting mixture was stirred under an Ar atmosphere for 5 min.

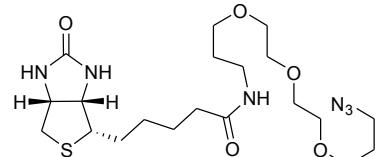
DMAP (9.6 mg, 0.079 mmol) was added and the reaction was allowed to stir at rt for 2 d. CH_2Cl_2 and H_2O were added and the reaction was cooled to 0 °C. 1M HCl was added until pH ~2 and the reaction was allowed to warm to rt and stir for 30 min. The reaction was extracted 4x with CH_2Cl_2 , washed with sat. $NaHCO_3$, H_2O , and sat. $NaCl$. The mixture was then dried over $MgSO_4$, filtered, and solvent was evaporated. The filtrate was purified by flash chromatography using a gradient elution of 100% EtOAc to 5% → 10% MeOH/EtOAc to give the methyl amide episilvestrol **6** (2.1 mg, 61%) as a white solid: $[\alpha]_D^{26} -173.8^\circ$ (*c* 0.11, CH_2Cl_2); IR ν_{max} (film): 3407, 2932, 1612, 1513, 1498, 1465, 1452, 1430, 1332, 1299, 1248, 1220, 1167, 1132, 1093, 1065, 1031, 985, 943, 902, 884, 827, 700 cm^{-1} ; 1H NMR (600 MHz) δ 7.10-7.04 (m, 5H), 6.96 (d, *J* = 7.0 Hz, 2H), 6.63 (d, *J* = 8.9 Hz, 2H), 6.43 (d, *J* = 1.6 Hz, 1H), 6.28 (d, *J* = 1.6 Hz, 1H), 6.00 (dd, *J* = 9.5, 4.9 Hz, 1H), 5.30 (s, 1H), 4.96 (d, *J* = 6.1 Hz, 1H), 4.60 (s, 1H), 4.35 (d, *J* = 12.3 Hz, 1H), 4.13 (ddd, *J* = 10.4, 7.6, 2.5 Hz, 1H), 4.00 (t, *J* = 11.2 Hz, 2H), 3.86 (s, 3H), 3.81 (dd, *J* = 11.6, 2.3 Hz, 1H), 3.69 (s, 3H), 3.68-3.61 (m, 4H), 3.51 (s, 3H), 2.67 (d, *J* = 4.8 Hz, 3H); ^{13}C NMR (150MHz) δ 171.1, 161.0, 159.9, 158.9, 157.4, 137.0, 129.0, 128.4, 128.1, 126.9, 126.8, 112.8, 108.7, 101.9, 95.4, 94.4, 93.7, 92.9, 92.5, 79.7, 71.6, 67.2, 62.0, 56.1, 55.8, 55.3, 51.2, 29.9, 26.6; HRMS (ESI): Calculated for $C_{34}H_{39}NNaO_{12} [M+Na]^+$ 676.2364, found 676.2361.



Propargyl amide 7. To a solution of episilvestric acid **5** (20.0 mg, 0.031 mmol) in CH_2Cl_2 (7 mL) at 0 °C was added DCC (44.5 mg, 0.216 mmol) and propargyl amine (15 μ L, 0.234 mmol), the resulting mixture was stirred under an Ar atmosphere for 5 min.

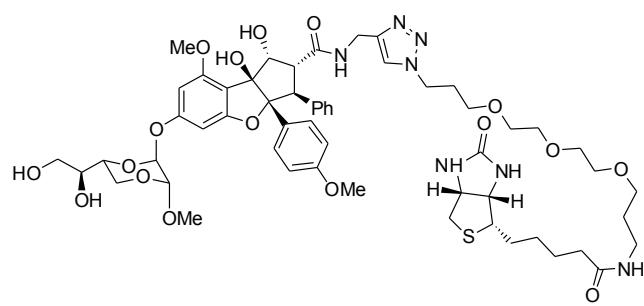
DMAP (24.5 mg, 0.201 mmol) was added and the reaction was allowed to stir at rt for 2 d. CH_2Cl_2 and H_2O were added and the reaction was cooled to 0 °C. 1M HCl was added until pH ~2 and the reaction was allowed to warm to rt and stir for 30 min. The reaction was

extracted 4x with CH₂Cl₂, washed with sat. NaHCO₃, H₂O, and sat. NaCl. The mixture was then dried over MgSO₄, filtered, and solvent was evaporated. The filtrate was purified by flash chromatography with 80% EtOAc/petrol as eluent to give the propargyl amide **7** (13.8 mg, 65%) as a white solid: $[\alpha]_D^{23} -88.1^\circ$ (*c* 0.40, CH₂Cl₂); IR ν_{max} (film): 3426, 2970, 2924, 1739, 1607, 1514, 1498, 1454, 1428, 1366, 1299, 1228, 1217, 1170, 1132, 1094, 1063, 1031, 986, 946, 904, 886, 825, 751, 734, 700, 660 cm⁻¹; ¹H NMR (600 MHz) δ 7.09-7.05 (m, 5H), 6.95 (d, *J* = 6.9 Hz, 2H), 6.63 (d, *J* = 8.9 Hz, 2H), 6.44 (d, *J* = 1.7 Hz, 1H), 6.34 (t, *J* = 5.0 Hz, 1H), 6.27 (d, *J* = 1.7, 1H), 5.29 (s, 1H), 4.98 (dd, *J* = 6.1, 2.1 Hz, 1H), 4.60 (s, 1H), 4.35 (d, *J* = 12.1 Hz, 1H), 4.12 (ddd, *J* = 10.6, 7.5, 2.7 Hz, 1H), 3.99 (t, *J* = 11.3 Hz, 1H), 3.95 (brs, 1H), 3.94 (ddd, *J* = 17.6, 6.8, 2.6 Hz, 1H), 3.87 (s, 3H), 3.84 (dd, *J* = 4.9, 2.6 Hz, 1H), 3.82-3.79 (m, 1H), 3.70 (dd, *J* = 12.8, 6.1 Hz, 1H), 3.69 (s, 3H), 3.67-3.64 (m, 2H), 3.63-3.60 (m, 1H), 3.50 (s, 3H), 2.54-2.50 (m, 2H), 2.15 (t, *J* = 2.5 Hz, 1H); ¹³C NMR (150MHz) δ 170.2, 161.0, 160.0, 158.9, 157.4, 136.8, 129.0, 128.4, 128.1, 126.9, 126.7, 112.8, 108.5, 101.8, 95.4, 94.4, 93.7, 92.9, 92.5, 79.5, 79.3, 71.9, 71.5, 67.2, 62.0, 60.2, 56.1, 55.8, 55.25, 55.23, 51.1, 29.6; HRMS (ESI): Calculated for C₃₆H₄₀NO₁₂ [M+H]⁺ 678.2545, found 678.2553. Propargyl amide **7** was pure by HPLC (*R*_t = 9.26 mins. 5 μ m Phenomenex Lux Cellulose-1 10x250mm column, 50% MeCN/H₂O eluent, flow rate: 2.00 mL/min).



Azide biotin linker 8. Azide biotin linker **8** was prepared in 3 steps from biotin as previously reported¹ with a modification as detailed below. Biotin (1.00 g, 4.093 mmol) was dissolved in 20 mL DMF at 70 °C and allowed to cool to rt. EtN₃ (0.73g, 1.00 mL, 7.175 mmol) was added, followed by pentafluorophenyl trifluoroacetate (1.63 g, 1.00 mL, 5.820 mmol). The reaction was allowed to stir for 30 min at rt and became pink. Solvent was removed in vacuo and the crude material was triturated with diethyl ether. The product biotin-pFp ester was recovered as a white solid (1.59 g, 95%). Biotin-pFp ester (1.20 g, 2.924 mmol) was dissolved in 40 mL DMF and cooled to 0 °C. A flask containing 4,7,10-tioxa-1,13-tridecanediamine (3.76 g, 3.75 mL, 17.107 mmol) and EtN₃ (0.58 g, 800 μ L, 5.740 mmol) was also cooled to 0 °C. Biotin-pFp ester was then transferred dropwise via cannula over 15 min into the diamine flask. The reaction was allowed to stir at rt for 1 h and became a pale pink. Solvent was removed in vacuo and the crude material was triturated with diethyl ether rather than purified by column chromatography as reported. This gave very pure biotin-amine as a white solid (1.23 g, 94%).

The biotin-amine (179.3 mg, 0.402 mmol) was dissolved in 7.0 mL MeOH. K₂CO₃ (83.4 mg, 0.603 mmol), CuSO₄·5H₂O, and imidazole-1-sulfonyl azide hydrochloride² (105.0 mg, 0.501 mmol) were added. The reaction was allowed to stir at rt for 16 h. Solvent was removed in vacuo and the crude material was purified by flash chromatography using a gradient elution of 15% → 25% MeOH/EtOAc to give the biotin azide linker **8** (103 mg, 55%, 58% bsrm) as a white, waxy solid: [α]_D²⁵ +34.9° (c 1.60, MeOH); IR ν_{max} (film): 3738, 3299, 2936, 2095, 1740, 1706, 1366, 1275, 1262, 1217, 1120, 764, 751 cm⁻¹; ¹H NMR (500 MHz, *d*₄-methanol) δ 4.49 (ddd, *J* = 7.8, 4.9, 0.6 Hz, 1H), 4.31 (dd, *J* = 7.9, 4.5 Hz, 1H), 3.65-3.63 (m, 4H), 3.61-3.58 (m, 4H), 3.56 (t, *J* = 6.1 Hz, 2H), 3.52 (t, *J* = 6.2 Hz, 2H), 3.40 (t, *J* = 6.7 Hz, 2H), 3.26 (t, *J* = 6.8 Hz, 2H), 3.21 (ddd, *J* = 7.9, 4.6, 3.5 Hz, 1H), 2.71 (d, *J* = 12.7 Hz, 1H), 2.20 (t, *J* = 7.4 Hz, 2H), 1.85-1.56 (m, 8H), 1.47-1.41 (m, 2H); ¹³C NMR (125MHz) δ 175.9, 166.0, 71.52, 71.51, 71.26, 71.22, 69.9, 68.9, 63.3, 61.6, 57.0, 49.5, 41.0, 39.7, 37.8, 36.9, 30.4, 30.2, 29.8, 29.5, 26.9; HRMS (ESI): Calculated for C₂₀H₃₆N₆NaO₅S [M+Na]⁺, 495.2360, found 495.2348. This compound was spectroscopically identical to that previously reported.¹



Biotinylated episilvestrol **9.** To propargyl amide **7** (3.5 mg, 0.005 mmol) in 2:1 DMSO: H₂O (1.5 mL) was added biotin azide **8** (3.2 mg, 0.007 mmol), TBTA (0.072 mg, 0.00014 mmol), and sodium ascorbate (0.276 mg, 0.0014 mmol). The reaction was wrapped in foil and CuSO₄ (0.02 mg, 0.00014 mmol) was added and the reaction was allowed to stir at 40 °C for 2 d. The solvent was evaporated and the mixture was purified via flash chromatography using a gradient elution of 100% EtOAc to 10% → 30% MeOH/EtOAc to give biotinylated episilvestrol **9** (5.1 mg, 86%, 95% bsrm) as a white solid: [α]_D²⁵ -79.4° (c 0.11, CH₂Cl₂); IR ν_{max} (film): 3310, 3083, 2970, 2928, 2875, 1739, 1700, 1647, 1623, 1541, 1515, 1498, 1455, 1434, 1366, 1301, 1228, 1217, 1169, 1133, 1119, 1097, 1067, 1031, 974, 946, 909, 887, 827, 731, 702 cm⁻¹; ¹H NMR (500 MHz) δ 7.75 (t, *J* = 5.2 Hz, 1H), 7.36 (s, 1H), 7.09 (d, *J* = 8.8 Hz, 3H), 7.05-6.99 (m, 6H), 6.73 (t, *J* = 5.0 Hz, 1H), 6.61 (d, *J* = 13.5 Hz, 2H), 6.43 (d, *J* = 1.6 Hz, 1H), 6.34 (brs, 1H), 6.24 (d, *J* = 1.6 Hz, 1H), 5.30 (s, 1H), 5.13 (brs, 1H), 4.86 (dd, *J* = 5.0, 2.1 Hz, 1H), 4.60 (s, 1H), 4.51 (brs, 1H), 4.47 (dd, *J* = 15.8, 6.1 Hz, 1H), 4.43 (dd, *J* = 7.0, 4.7 Hz, 1H), 4.41 (d, *J* = 14.0 Hz, 1H), 4.34, (t, *J* = 6.9 Hz, 3H), 4.28 (dd, *J* = 7.4, 4.7 Hz, 1H), 4.09 (ddd, *J* = 10.6, 7.9, 2.5

Hz, 1H), 3.97 (t, J = 11.3, 1H), 3.87 (dd, J = 13.8, 5.2 Hz, 1H), 3.84-3.79 (m, 2H), 3.81 (s, 3H), 3.74 (m, 1H), 3.69-3.65 (m, 2H), 3.67 (s, 3H), 3.62-3.51 (m, 16H), 3.50 (s, 3H), 3.40-3.25 (m, 6H), 3.12 (ddd, J = 11.7, 7.3, 4.6 Hz, 1H), 2.86 (dd, J = 12.9, 5.0 Hz, 1H), 2.65 (d, J = 12.8 Hz, 1H), 2.45 (brs, 1H), 2.10-2.02 (m 5H), 1.73-1.54 (m, 21 H), 1.45-1.32 (m, 3H), 1.25 (s, 3H); ^{13}C NMR (125MHz) δ 173.2, 171.2, 163.8, 161.0, 159.6 158.6, 157.6, 144.87, 144.85, 137.0, 128.9, 128.4, 127.8, 127.0, 126.5, 123.0, 112.6, 108.5, 101.9, 95.4, 94.6, 93.7, 93.5, 92.1, 79.0, 71.5, 70.5, 70.4, 70.2, 70.0, 69.9, 67.2, 67.1, 62.2, 62.1, 60.4, 60.1, 56.1, 55.8, 55.7, 55.10, 55.09, 51.8, 47.0, 40.6, 37.7, 35.6, 35.0, 30.1, 29.7, 28.8, 28.21, 28.16, 25.5; HRMS (ESI): Calculated for $\text{C}_{56}\text{H}_{75}\text{N}_7\text{NaO}_{17}\text{S} [\text{M}+\text{Na}]^+$ 1172.4832, found 1172.4846. Biotinylated episilvestrol **9** was pure by HPLC (R_t = 8.43 mins. 5 μm , Phenomenex Lux cellulose-1 10 x 250mm column, 40% MeCN/H₂O eluent, flow rate: 2.00 mL/min).

In vitro translations

In vitro translations were performed for 1 h at 30 °C in rabbit reticulocyte lysate essentially as described.³ The Dual Glo Luciferase Assay kit (Promega, Madison, WI, USA) was used to measure Firefly and Renilla luciferase activity, translated from capped mRNA transcribed from pSP/(CAG)₃₃/FF/HCV/Ren.pA51. Data are represented as Firefly luciferase activity/Renilla luciferase activity relative to vehicle (DMSO-treated) controls. Renilla luciferase serves as an internal control since HCV IRES-driven translation is resistant to silvestrol.⁴

Cell viability assay

Mouse embryonic fibroblasts (MEFs) immortalized with SV40 large T antigen (1000 cells/well in a 96 well opaque plate) were seeded into serial dilutions of compound in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (FCS; Bovogen, East Keilor, Australia), 50 μM 2-mercaptoethanol (Sigma, Castle Hill, Australia) and 100 μM asparagine (Sigma). After 72 h at 37 °C/10 % CO₂, the number of viable cells was measured as a function of ATP concentration using the CellTiter-Glo Luminescent Cell Viability Assay (Promega, Alexandria, Australia).

Rate of translation in MEFs

MEFs (45 000 cells/well) were seed in 24h well plates. Once cells adhered, media was replaced with indicated concentrations of compound for 40 minutes at 37 °C/10 % CO₂, after

which 5 μ L of [35 S]Easy Tag Express Protein Labeling mix (1175 Ci/mmol) (Perkin Elmer, Waltham, MA, USA) was added for an additional 20 minutes. Cells were then washed with PBS, and subsequently lysed in Ripa buffer. Half the lysate was spotted onto Whatman paper preblocked with L-methionine and protein was precipitated with TCA before quantitation by Scintillation counting.⁴ The rate of translation was standardised to protein content using the Micro BCA protein assay (Pierce, Scoresby, Australia).

Streptavidin pulldowns

MEFs (10 million cells per sample) were washed in PBS and lysed in 0.5 mL of IP buffer [50 mM Tris pH 7.5, 120mM NaCl, 2 mM EDTA, 2 mM KCl, 1% Triton X-100, protease inhibitors (complete mini EDTA-free protease inhibitor cocktail tablet; Roche)] and membranes were cleared by centrifugation. RNase A (Sigma) was added (10 μ g) to the lysates and were incubated at room temperature for 10 min to reduce non-specific pulldown of RNA-binding proteins. Samples were precleared with streptavidin sepharose high performance beads (GE Healthcare, Uppsala, Sweden) for 30 min at 4 °C. Biotinylated episilvestrol (9) or biotin alone (AK Scientific) (20 μ M) was then added to lysates with fresh streptavidin sepharose beads and rotated end-over-end overnight at 4 °C. Pulldowns were washed in IP buffer 5 times at 4 °C and eluted with successive treatments of 50 μ M silvestrol, 25mM glycine pH 2.5, at room temperature and finally in LDS (NuPAGE® LDS Sample Buffer 1X, Invitrogen, CA, USA) at 95 °C. Equal volumes of the samples were separated on 4-12% NuPAGE® gels, transferred to PVDF and probed using the following antibodies: eIF4AI (ab31217; Abcam), eIF4AII (ab31218; Abcam), eIF4AIII (ab32485; Abcam), eIF4G (C45A4; Cell Signaling), and eIF4E (87; BD Transduction Laboratories), DDX3 (D19B4; Cell Signalling), DHX29 (D19E10; Cell Signalling). Alternatively, samples were silver stained essentially as described.⁵

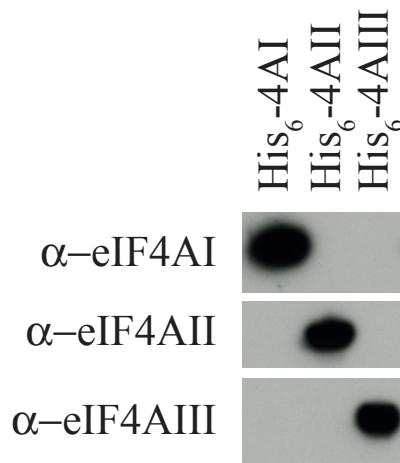


Figure 1. Specificity of indicated antibodies. Recombinant His₆-tagged protein (100ng) was separated by NuPAGE®, transferred to PVDF, and probed with antibodies against eIF4AI (ab31217; Abcam), eIF4AII (ab31218; Abcam), and eIF4AIII (ab32485; Abcam). Recombinant proteins were purified as previously described.

Protein Mass Spectroscopy

Eluted proteins were digested by the filter-aided sample preparation method.⁶ Briefly, the eluate was added to SDS-containing buffer and loaded onto a Vivaspin 500 30 K device (Sartorius Stedman). SDS was removed by urea exchange followed by alkylation with 50 mM iodoacetamide for 20 min. Urea was then replaced with 20 mM ammonium bicarbonate before the proteins were digested overnight at 37 °C with trypsin (Gold, Promega) (1 µg of trypsin:100 µg of protein). The peptides were collected from the filter by centrifugation followed by two additional elutions with 20 mM ammonium bicarbonate. Peptide eluates were then quantified on a Nanodrop 2000 at a wavelength of 280 nm and acidified to 1% formic acid prior to MS analysis.

MS data were acquired on an LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific) fitted with a Nano-ESI source (Proxeon; Thermo Fisher Scientific) coupled to a nano-HPLC system (Agilent Technologies). Approximately 1 µg of total digested peptide was loaded onto a 20 mm long nanoACQUITY pre-column with 180 µm I.D. and 5 µm C18 silica bead (Waters) in 5% buffer B at a flow rate of 10ul/min (Buffer A - 0.1% formic acid. Buffer B - 90% ACN, 0.1% formic acid) and then resolved on a 100 mm nanoACQUITY column with 100 µm I.D. and 1.7 µm C18 silica bead (Waters) using a 70 min gradient (5% to 45% buffer B) at a flow rate of 1.2 µl/min. The Orbitrap was then run in a data-dependent

acquisition mode with the Orbitrap resolution set at 60,000 and the top-five multiply charged species selected for fragmentation in the linear ion trap by collision-induced dissociation (single charged species were ignored). The ion threshold was set to 15,000 counts for MS/MS. The activation time was set to 30 ms. Raw files consisting of full-scan MS and ion-trap spectra were converted to the MGF data format and searched against an in-house generated target/decoy database (LudwigNR-Q412, from WEHI Bioinformatics) and searched using our in-house Mascot Cluster (WEHI Systems Biology Mascot Server, Matrix Science). Spectra were searched with a mass tolerance of 10 ppm in MS mode and 0.7 Da in MS/MS mode, allowing up to 3 missed cleavage sites. Cysteine carbamidomethylation was included as a fixed modification, while oxidized Methionine and Lysine acetylation were set to variable. Peptides were identified at a 1% false discovery rate and were visualized using Scaffold 3.0 (Proteome Software).

Silvestrol Elutions, Samples report created on 11/12/2012
 Experiment: Silvestrol Elutions
 Peak List Generator: unknown
 Version: unknown
 Charge States Calculated: unknown
 Taxonomy: Mus musculus
 Number of Proteins: 96518
 Does database contain common contaminants?: unknown
 Search Engine Set: 1 Search Engine
 Search Engine: Mascot
 Version: 2.3.0.05
 Samples: All Samples
 Fragment Tolerance: 0.70 Da (Monoisotopic)
 Parent Tolerance: 10.0 PPM (Monoisotopic)
 Fixed Modifications: +57 m/z C (Carbamidomethyl)
 Variable Modifications: +16 m/z M (Oxidation)
 Database: The LudwigNR_Q312_generic_forward database (selected for Mus musculus, unknown version, 96518 entries)
 Digestion Enzyme: Trypsin
 Missed Cleavages: 3

Scaffold Version Scaffold 3.6.1
 Peptide Thresholds: 90.0% minimum
 Protein Thresholds: 95.0% minimum and 1 peptides minimum

Elutions #	Number of Unique Peptides Visible?	Starred?	Identified Proteins (80)	Accession Number	Molecular Weight	Protein Grouping Ambiguity	Quantitative Variance	Taxonomy	Uncategorized Sample							
									Blank 1	Biotin only, silvestrol elution	Blank 2	Blank 3	Blank 4			
1	TRUE	FALSE	sp P60843 Eukaryotic initiation factor 1 Tax_id=10090 [Mus musculus]	P60843	46 kDa			unknown	0	0	0	15	0	0	0	26
1.1	TRUE	FALSE	sp Q4FZL1 Eif4f1 protein (Fragment) Tax_id=10090 [Mus musculus]	Q4FZL1	46 kDa			unknown	0	0	0	15	0	0	0	26
2	TRUE	FALSE	sp P60710 Actin, cytoplasmic 2 Tax_id=10090 [Mus musculus]	P60710	42 kDa	TRUE		unknown	0	10	0	2	0	6	0	10
2.1	TRUE	FALSE	sp P63260 Actin, cytoplasmic 2 Tax_id=10090 [Mus musculus]	P63260	42 kDa	TRUE		unknown	0	10	0	2	0	6	0	10
2.2	TRUE	FALSE	sp Q3U5R4 Putative uncharacterized protein Tax_id=10090 [Mus musculus]	Q3U5R4	42 kDa	TRUE		unknown	0	10	0	2	0	6	0	10
2.3	TRUE	FALSE	sp Q3UAF6 Putative uncharacterized protein Tax_id=10090 [Mus musculus]	Q3UAF6	42 kDa	TRUE		unknown	0	10	0	2	0	6	0	10
2.4	TRUE	FALSE	sp Q3UAF7 Putative uncharacterized protein Tax_id=10090 [Mus musculus]	Q3UAF7	42 kDa	TRUE		unknown	0	10	0	2	0	6	0	10
3	TRUE	FALSE	sp P60720 Actin, cytoplasmic 1 Tax_id=10090 [Mus musculus]	P60720	36 kDa			unknown	0	4	0	0	0	8	0	11
3.1	TRUE	FALSE	sp F0G172 Uncharacterized protein (fragment) Tax_id=10090 [Mus musculus]	F0G172	30 kDa			unknown	0	4	0	0	0	8	0	11
3.2	TRUE	FALSE	sp P129706 Ribosomal protein L7a Tax_id=10090 [Mus musculus]	P12970	30 kDa			unknown	0	4	0	0	0	8	0	11
3.3	TRUE	FALSE	sp Q6P1A9 Ribosomal protein L7a Tax_id=10090 [Mus musculus]	Q6P1A9	30 kDa			unknown	0	4	0	0	0	8	0	11
3.4	TRUE	FALSE	sp Q800717 Ribosomal protein (Fragment) Tax_id=10090 [Mus musculus]	Q800717	30 kDa			unknown	0	4	0	0	0	8	0	11
4	TRUE	FALSE	sp P62702 0S ribosomal protein S4, X isoform Tax_id=10090 [Mus musculus]	P62702	30 kDa			unknown	0	10	0	2	0	5	0	7
4.1	TRUE	FALSE	sp Q545F8 Uncharacterized protein Tax_id=10090 [Mus musculus]	Q545F8	27 kDa			unknown	0	10	0	2	0	5	0	7
5	TRUE	FALSE	sp P43277 Histone H1 Tax_id=10090 [Mus musculus]	P43277	22 kDa	TRUE		unknown	0	5	0	1	0	10	0	7
5.1	TRUE	FALSE	sp Q3U292 Putative uncharacterized protein Tax_id=10090 [Mus musculus]	Q3U292	22 kDa	TRUE		unknown	0	5	0	1	0	10	0	7
6	TRUE	FALSE	sp D3Z6C3 Uncharacterized protein Tax_id=10090 [Mus musculus]	D3Z6C3	30 kDa			unknown	0	10	0	0	0	4	0	8
6.1	TRUE	FALSE	sp P97354 Ribosomal protein S3a Tax_id=10090 [Mus musculus]	P97354	30 kDa			unknown	0	10	0	0	0	4	0	8
6.2	TRUE	FALSE	sp Q3UAC2 Putative uncharacterized protein Tax_id=10090 [Mus musculus]	Q3UAC2	30 kDa			unknown	0	10	0	0	0	4	0	8
7	TRUE	FALSE	sp P60717 Ribosomal protein L1 Tax_id=10090 [Mus musculus]	P60717	27.5 kDa			unknown	0	2	0	0	0	13	0	9
7.1	TRUE	FALSE	sp P60717 Ribosomal protein S18 Tax_id=10090 [Mus musculus]	P60717	18 kDa			unknown	0	5	0	1	0	6	0	8
8	TRUE	FALSE	sp Q5VSZ5 MCG116771 Tax_id=10090 [Mus musculus]	Q5VSZ5	18 kDa			unknown	0	5	0	1	0	6	0	8
9	TRUE	FALSE	sp P62301 0S ribosomal protein S13 Tax_id=10090 [Mus musculus]	P62301	17 kDa			unknown	0	6	0	1	0	4	0	6
10	TRUE	FALSE	sp D3YWP3 MCG103428 Tax_id=10090 [Mus musculus]	D3YWP3	18 kDa			unknown	0	6	0	0	0	7	0	5
10.1	TRUE	FALSE	sp P62751 0S ribosomal protein L23a Tax_id=10090 [Mus musculus]	P62751	18 kDa			unknown	0	6	0	0	0	7	0	5
10.2	TRUE	FALSE	sp Q4VX9X9 Ribosomal protein L23a Tax_id=10090 [Mus musculus]	Q4VX9X9	18 kDa			unknown	0	6	0	0	0	7	0	5
10.3	TRUE	FALSE	sp Q9Y1K6 Ribosomal protein (Fragment) Tax_id=10090 [Mus musculus]	Q9Y1K6	17 kDa			unknown	0	6	0	0	0	7	0	5
10.4	TRUE	FALSE	sp XP_001476183 Predicted: 60S ribosomal protein L23a-like Tax_id=10090 [Mus musculus]	XP_001476183	18 kDa			unknown	0	6	0	0	0	7	0	5
11	TRUE	FALSE	sp Q5I438 Nucleophosmin Tax_id=10090 [Mus musculus]	Q5I438	33 kDa			unknown	0	8	0	1	0	4	0	3
11.1	TRUE	FALSE	sp Q0I937 Nucleophosmin Tax_id=10090 [Mus musculus]	Q0I937	33 kDa			unknown	0	8	0	1	0	4	0	3
11.2	TRUE	FALSE	sp Q5I473 Nucleophosmin Tax_id=10090 [Mus musculus]	Q5I473	33 kDa			unknown	0	8	0	1	0	4	0	3
12	TRUE	FALSE	sp P60717 Ribosomal protein L1 Tax_id=10090 [Mus musculus]	P60717	34 kDa			unknown	0	8	0	2	0	3	0	3
12.1	TRUE	FALSE	sp Q3T8K3 Ribosomal protein L1 Tax_id=10090 [Mus musculus]	Q3T8K3	34 kDa			unknown	0	8	0	2	0	3	0	3
12.2	TRUE	FALSE	sp Q3U850 Putative uncharacterized protein Tax_id=10090 [Mus musculus]	Q3U850	34 kDa			unknown	0	8	0	2	0	3	0	3
13	TRUE	FALSE	sp P61255 Ribosomal protein L26 Tax_id=10090 [Mus musculus]	P61255	17 kDa			unknown	0	4	0	1	0	5	0	4
14	TRUE	FALSE	sp P379116 Ribosomal protein L6 Tax_id=10090 [Mus musculus]	P37911	33 kDa			unknown	0	7	0	1	0	8	0	3
14.2	TRUE	FALSE	sp XP_483949 Predicted: 60S ribosomal protein L6-like Tax_id=10090 [Mus musculus]	XP_483949	35 kDa			unknown	0	7	0	1	0	8	0	3
15	TRUE	FALSE	sp Q3W40 Putative uncharacterized protein Tax_id=10090 [Mus musculus]	Q3W40	74 kDa			unknown	0	0	0	0	0	11	0	4
15.1	TRUE	FALSE	sp Q5T200 MCG1193 Protein (Fragment) Tax_id=10090 [Mus musculus]	Q5T200	75 kDa			unknown	0	0	0	0	0	11	0	4
15.2	TRUE	FALSE	sp Q9D0E1 Heterogeneous nuclear ribonucleoprotein M Tax_id=10090 [Mus musculus]	Q9D0E1	?			unknown	0	0	0	0	0	11	0	4
15.3	TRUE	FALSE	sp Q9D0E1-2 Heterogeneous nuclear ribonucleoprotein M Tax_id=10090 [Mus musculus]	Q9D0E1-2	74 kDa			unknown	0	0	0	0	0	11	0	4
16	TRUE	FALSE	sp E3W5040 Uncharacterized protein Tax_id=10090 [Mus musculus]	E3W5040	48 kDa			unknown	0	5	0	1	0	2	0	3
16.1	TRUE	FALSE	sp P61230 0S ribosomal protein L13a Tax_id=10090 [Mus musculus]	P61230	23 kDa			unknown	0	5	0	1	0	2	0	3
17	TRUE	FALSE	sp P60716 0S ribosomal protein L13a Tax_id=10090 [Mus musculus]	P60716	21 kDa			unknown	0	4	0	1	0	5	0	2
18	TRUE	FALSE	sp P60716 0S ribosomal protein L13a Tax_id=10090 [Mus musculus]	P60716	14 kDa			unknown	0	3	0	0	0	4	0	4
18.1	TRUE	FALSE	sp XP_03036045 Predicted: 60S ribosomal protein L132-kDa Tax_id=10090 [Mus musculus]	XP_03036045	16 kDa			unknown	0	3	0	0	0	4	0	4
19	TRUE	FALSE	sp D3YX54 Ribosomal protein L13 Tax_id=10090 [Mus musculus]	D3YX54	24 kDa			unknown	0	4	0	2	0	5	0	3
19.1	TRUE	FALSE	sp P62301 0S ribosomal protein L13 Tax_id=10090 [Mus musculus]	P62301	24 kDa			unknown	0	4	0	2	0	5	0	3
19.2	TRUE	FALSE	sp XP_001474740 Predicted: 60S ribosomal protein L13-kDa Tax_id=10090 [Mus musculus]	XP_001474740	24 kDa			unknown	0	4	0	2	0	5	0	3
20	TRUE	FALSE	sp P629006 Ribosomal protein L13 Tax_id=10090 [Mus musculus]	P62900	14 kDa			unknown	0	3	0	1	0	3	0	5
20.1	TRUE	FALSE	sp Q9CY93 Putative uncharacterized protein Tax_id=10090 [Mus musculus]	Q9CY93	14 kDa			unknown	0	3	0	1	0	3	0	5
20.2	TRUE	FALSE	sp XP_0229881 Predicted: 60S ribosomal protein L13-like isoform 1 Tax_id=10090 [Mus musculus]	XP_0229881	14 kDa			unknown	0	3	0	1	0	3	0	5
21	TRUE	FALSE	sp D3Y41605 Ribosomal protein L36 Tax_id=10090 [Mus musculus]	D3Y41605	12 kDa			unknown	0	3	0	0	0	3	0	4
21.1	TRUE	FALSE	sp D3J3R1605 Ribosomal protein L36 Tax_id=10090 [Mus musculus]	D3J3R16	12 kDa			unknown	0	3	0	0	0	3	0	4
21.2	TRUE	FALSE	sp Q5M0U1 0S ribosomal protein L13 Tax_id=10090 [Mus musculus]	Q5M0U1	12 kDa			unknown	0	3	0	0	0	3	0	4
21.3	TRUE	FALSE	sp Q5M0U1 0S ribosomal protein L13 Tax_id=10090 [Mus musculus]	Q5M0U1	12 kDa			unknown	0	3	0	0	0	3	0	4
22	TRUE	FALSE	sp P607203 0S ribosomal protein L13 Tax_id=10090 [Mus musculus]	P607203	14 kDa			unknown	0	4	0	1	0	2	0	4
22.1	TRUE	FALSE	sp Q3U361 Ribosomal protein Tax_id=10090 [Mus musculus]	Q3U361	25 kDa			unknown	0	1	0	0	0	4	0	4
23	TRUE	FALSE	sp Q5XJ60 Ribosomal protein Tax_id=10090 [Mus musculus]	Q5XJ60	25 kDa			unknown	0	1	0	0	0	4	0	4
24	TRUE	FALSE	sp Q551426 Ribosomal protein L13a Tax_id=10090 [Mus musculus]	Q551426	13 kDa			unknown	0	3	0	0	0	3	0	3
24.1	TRUE	FALSE	sp Q8OBV7 Putative uncharacterized protein Tax_id=10090 [Mus musculus]	Q8OBV7	13 kDa			unknown	0	3	0	0	0	3	0	3
25	TRUE	FALSE	sp P61358 Ribosomal protein L27 Tax_id=10090 [Mus musculus]	P61358	16 kDa			unknown	0	5	0	0	0	2	0	4
26	TRUE	FALSE	sp P61105 Ribosomal protein L28 Tax_id=10090 [Mus musculus]	P61105	16 kDa			unknown	0	3	0	1	0	3	0	2
26.1	TRUE	FALSE	sp Q5MN95 Ribosomal protein L28 Tax_id=10090 [Mus musculus]	Q5MN95	16 kDa			unknown	0	3	0	1	0	3	0	2
26.2	TRUE	FALSE	sp XP_014739741 Predicted: 60S ribosomal protein L28-like Tax_id=10090 [Mus musculus]	XP_014739741	16 kDa			unknown	0	3	0	1	0	3	0	2
27	TRUE	FALSE	sp E3PW19 Ribosomal protein L15 Tax_id=10090 [Mus musculus]	E3PW19	24 kDa			unknown	0	7	0	0	0	0	0	2
27.1	TRUE	FALSE	sp E3QAZ2 Ribosomal protein L15 Tax_id=10090 [Mus musculus]	E3QAZ2	24 kDa			unknown	0	7	0	0	0	0	0	2
27.2	TRUE	FALSE	sp Q5M2Q1 Ribosomal protein L15 Tax_id=10090 [Mus musculus]	Q5M2Q1	24 kDa			unknown	0	7	0	0	0	0	0	2
28	TRUE	FALSE	sp Q4VAC28 Ribosomal protein L15 Tax_id=10090 [Mus musculus]	Q4VAC28	19 kDa			unknown	0	5	0	0	0	1	0	2
28.1	TRUE	FALSE	sp Q5C9M8 MCG12046 Tax_id=10090 [Mus musculus]	Q5C9M8	19 kDa			unknown	0	5	0	0	0	1	0	2
29	TRUE	FALSE	sp P14869 0S acidic ribosomal protein P0 Tax_id=10090 [Mus musculus]	P14869	34 kDa			unknown	0	4	0	2	0	2	0	2
30																

37.3	TRUE	FALSE	ensENSMSU00000126334/ENSMUSG0000009544 transcript ENSMUST000126334	ENSMUSP00000126334	30 kDa	unknown	0	6	0	0	0	2	0	0	1
37.4	TRUE	FALSE	tr F6Y7Z Uncharacterized protein (Fragment) Tax_id=10990 [Mus musculus]	F6Y7Z4	30 kDa	unknown	0	6	0	0	0	2	0	0	1
37.5	TRUE	FALSE	sp P25444 405 ribosomal protein S2 Tax_id=10990 [Mus musculus]	P25444	31 kDa	unknown	0	6	0	0	0	2	0	0	1
37.6	TRUE	FALSE	tr Q3T120 Putative uncharacterized protein Tax_id=10990 [Mus musculus]	Q3T120	31 kDa	unknown	0	6	0	0	0	2	0	0	1
37.7	TRUE	FALSE	tr Q3T1LE Putative uncharacterized protein Tax_id=10990 [Mus musculus]	Q3T1LE	31 kDa	unknown	0	6	0	0	0	2	0	0	1
37.8	TRUE	FALSE	tr Q3T1XK Putative uncharacterized protein Tax_id=10990 [Mus musculus]	Q3T1XK	24 kDa	unknown	0	6	0	0	0	2	0	0	1
37.9	TRUE	FALSE	tr Q3T1XV Putative uncharacterized protein Tax_id=10990 [Mus musculus]	Q3T1XV	31 kDa	unknown	0	6	0	0	0	2	0	0	1
37.10	TRUE	FALSE	XP_003086745_1 PREDICTED: 405 ribosomal protein S2-like Tax_id=10990 [Mus musculus]	XP_003086745.1	31 kDa	unknown	0	6	0	0	0	2	0	0	1
38	TRUE	FALSE	sp P938E6 6405 ribosomal protein L1 Tax_id=10990 [Mus musculus]	P938E6	47 kDa	unknown	0	8	0	0	0	0	0	0	0
39	TRUE	FALSE	sp P96151 6406 ribosomal protein L175 Tax_id=10990 [Mus musculus]	P96151	10 kDa	unknown	0	3	0	0	0	2	0	0	2
39.1	TRUE	FALSE	tr G3M9N6 Rpl32a protein Tax_id=10990 [Mus musculus]	G3M9N6	9 kDa	unknown	0	3	0	0	0	2	0	0	2
39.2	TRUE	FALSE	tr Q9CYW3 Putative uncharacterized protein Tax_id=10990 [Mus musculus]	Q9CYW3	10 kDa	unknown	0	3	0	0	0	2	0	0	2
40	TRUE	FALSE	sp P62889 605 ribosomal protein L30 Tax_id=10990 [Mus musculus]	P62889	13 kDa	unknown	0	2	0	0	0	4	0	0	4
41	TRUE	FALSE	ensENSMSU00000102200/ENSMUSG00000086833 transcript ENSMUST000102200	ENSMUSP00000102200	12 kDa	unknown	0	3	0	0	0	2	0	0	2
41.1	TRUE	FALSE	tr FW3W4J1 Ribosomal protein S15A (Fragment) Tax_id=10990 [Mus musculus]	FW3W4J1	12 kDa	unknown	0	3	0	0	0	2	0	0	2
41.2	TRUE	FALSE	sp P62245 405 ribosomal protein S15a Tax_id=10990 [Mus musculus]	P62245	15 kDa	unknown	0	3	0	0	0	2	0	0	2
42	TRUE	FALSE	tr P9QJN0 Uncharacterized protein Tax_id=10990 [Mus musculus]	P9QJN0	18 kDa	unknown	0	4	0	0	0	2	0	0	1
42.2	TRUE	FALSE	tr Q3UW40 Putative uncharacterized protein Tax_id=10990 [Mus musculus]	Q3UW40	18 kDa	unknown	0	4	0	0	0	2	0	0	1
42.3	TRUE	FALSE	sp P08P676 605 ribosomal protein L24 Tax_id=10990 [Mus musculus]	P08P676	18 kDa	unknown	0	4	0	0	0	2	0	0	1
43	TRUE	FALSE	tr G3XAX1 Heterogeneous nuclear ribonucleoprotein U, isoform CRA, Tax_id=10990 [Mus musculus]	G3XAX10	87 kDa	unknown	0	5	0	0	0	1	0	0	0
43.1	TRUE	FALSE	tr Q3T1VE Putative uncharacterized protein Tax_id=10990 [Mus musculus]	Q3T1VE	88 kDa	unknown	0	5	0	0	0	1	0	0	0
43.2	TRUE	FALSE	tr Q3T1VH Putative uncharacterized protein Tax_id=10990 [Mus musculus]	Q3T1VH	88 kDa	unknown	0	5	0	0	0	1	0	0	0
43.3	TRUE	FALSE	tr Q3T1XW Putative uncharacterized protein Tax_id=10990 [Mus musculus]	Q3T1XW	88 kDa	unknown	0	5	0	0	0	1	0	0	0
43.4	TRUE	FALSE	tr Q3T1H5 Putative uncharacterized protein Tax_id=10990 [Mus musculus]	Q3T1H5	88 kDa	unknown	0	5	0	0	0	1	0	0	0
43.5	TRUE	FALSE	tr Q3C290 Putative uncharacterized protein Tax_id=10990 [Mus musculus]	Q3C290	88 kDa	unknown	0	5	0	0	0	1	0	0	0
43.6	TRUE	FALSE	tr Q8V3EK3 Heterocyste nucleic acid/neuroleptic 11 Tax_id=10990 [Mus musculus]	Q8V3EK3	88 kDa	unknown	0	5	0	0	0	1	0	0	0
44	TRUE	FALSE	tr D3YYQ0 Uncharacterized protein Tax_id=10990 [Mus musculus]	D3YYQ0	22 kDa	unknown	0	4	0	0	0	3	0	0	1
44.1	TRUE	FALSE	sp P51410 605 ribosomal protein L9 Tax_id=10990 [Mus musculus]	P51410	22 kDa	unknown	0	4	0	0	0	3	0	0	1
44.2	TRUE	FALSE	tr Q5M915 Ribosomal protein L9A Tax_id=10990 [Mus musculus]	Q5M915	22 kDa	unknown	0	4	0	0	0	3	0	0	1
45	TRUE	FALSE	tr Q6PH1Z1 Rpl17 protein Tax_id=10990 [Mus musculus]	Q6PH1Z1	21 kDa	unknown	0	6	0	0	0	2	0	0	0
46	TRUE	FALSE	sp P53979 605 ribosomal protein L12 Tax_id=10990 [Mus musculus]	P53979	18 kDa	unknown	0	3	0	1	0	2	0	0	2
46.1	TRUE	FALSE	tr Q3T1Q2 Putative uncharacterized protein Tax_id=10990 [Mus musculus]	Q3T1Q2	18 kDa	unknown	0	3	0	1	0	2	0	0	2
46.2	TRUE	FALSE	tr Q8C2K0 Putative uncharacterized protein Tax_id=10990 [Mus musculus]	Q8C2K0	18 kDa	unknown	0	3	0	1	0	2	0	0	2
46.3	TRUE	FALSE	refEXP_00100321 PREDICTED: 605 ribosomal protein L12-like Tax_id=10990 XP_00100321.1	XP_00100321.1	18 kDa	unknown	0	3	0	1	0	2	0	0	2
47	TRUE	FALSE	tr A2A547 Ribosomal protein L19 Tax_id=10990 [Mus musculus]	A2A547	23 kDa	unknown	0	4	0	1	0	1	0	0	0
47.1	TRUE	FALSE	sp P84099 605 ribosomal protein L19 Tax_id=10990 [Mus musculus]	P84099	23 kDa	unknown	0	4	0	1	0	1	0	0	0
48	TRUE	FALSE	tr P9P5A1 Uncharacterized protein Tax_id=10990 [Mus musculus]	P9P5A1	19 kDa	unknown	0	5	0	0	0	0	0	0	0
48.1	TRUE	FALSE	tr Q3T1VE Putative uncharacterized protein Tax_id=10990 [Mus musculus]	Q3T1VE	19 kDa	unknown	0	5	0	0	0	1	0	0	0
48.2	TRUE	FALSE	tr Q5P797 Putative uncharacterized protein Tax_id=10990 [Mus musculus]	Q5P797	19 kDa	unknown	0	5	0	0	0	0	0	0	0
49	TRUE	FALSE	tr P62264 Ribosomal protein L16 Tax_id=10990 [Mus musculus]	P62264	11 kDa	unknown	0	3	0	0	0	2	0	0	2
50	TRUE	FALSE	tr A0A1UV1 Histone H2A (Fragment) Tax_id=10990 [Mus musculus]	A0A1UV1	14 kDa	unknown	0	3	0	0	0	1	0	0	1
50.1	TRUE	FALSE	tr FW3W8X Histone H2A (Fragment) Tax_id=10990 [Mus musculus]	FW3W8X	14 kDa	unknown	0	3	0	0	0	1	0	0	1
50.2	TRUE	FALSE	sp P22752 Histone H2A type I-H Tax_id=10990 [Mus musculus]	P22752	14 kDa	unknown	0	3	0	0	0	1	0	0	1
50.3	TRUE	FALSE	tr D64426 Histone H2A (Fragment) Tax_id=10990 [Mus musculus]	D64426	15 kDa	unknown	0	3	0	0	0	1	0	0	1
50.4	TRUE	FALSE	sp Q64523 Histone H2A type 2-C Tax_id=10990 [Mus musculus]	Q64523	14 kDa	unknown	0	3	0	0	0	1	0	0	1
50.5	TRUE	FALSE	sp Q6GSS7 Histone H2A type 2-A Tax_id=10990 [Mus musculus]	Q6GSS7	14 kDa	unknown	0	3	0	0	0	1	0	0	1
50.6	TRUE	FALSE	tr Q8BFU2 Histone H2A type 3 Tax_id=10990 [Mus musculus]	Q8BFU2	14 kDa	unknown	0	3	0	0	0	1	0	0	1
50.7	TRUE	FALSE	tr Q8C2P4 Histone H2A Tax_id=10990 [Mus musculus]	Q8C2P4	14 kDa	unknown	0	3	0	0	0	1	0	0	1
50.8	TRUE	FALSE	tr Q8CPGP1 Histone H2A type 1-H Tax_id=10990 [Mus musculus]	Q8CPGP1	14 kDa	unknown	0	3	0	0	0	1	0	0	1
50.9	TRUE	FALSE	tr Q8CPGP6 Histone H2A type 1-B Tax_id=10990 [Mus musculus]	Q8CPGP6	14 kDa	unknown	0	3	0	0	0	1	0	0	1
50.10	TRUE	FALSE	tr Q8CPGP7 Histone H2A type 1-K Tax_id=10990 [Mus musculus]	Q8CPGP7	14 kDa	unknown	0	3	0	0	0	1	0	0	1
50.11	TRUE	FALSE	tr Q8R1M2 Histone H2A Tax_id=10990 [Mus musculus]	Q8R1M2	14 kDa	unknown	0	3	0	0	0	1	0	0	1
50.12	TRUE	FALSE	refEXP_003084967_1 PREDICTED: 3c substrate contacta Tax_id=10990 [Mus musculus]	XP_003084967.1	32 kDa	unknown	0	3	0	0	0	1	0	0	0
51	TRUE	FALSE	sp P50496 405 ribosomal protein S16 Tax_id=10990 [Mus musculus]	P50496	32 kDa	unknown	0	6	0	0	0	1	0	0	0
51.1	TRUE	FALSE	tr Q5C769 Putative protein S16 Tax_id=10990 [Mus musculus]	Q5C769	19 kDa	unknown	0	6	0	0	0	1	0	0	0
51.2	TRUE	FALSE	tr Q641N2 Rpl16a (Fragment) Tax_id=10990 [Mus musculus]	Q641N2	18 kDa	unknown	0	6	0	0	0	1	0	0	0
51.3	TRUE	FALSE	tr Q6P264 605 ribosomal protein L23 Tax_id=10990 [Mus musculus]	P62264	18 kDa	unknown	0	6	0	0	0	1	0	0	0
51.4	TRUE	FALSE	sp P67084 605 ribosomal protein L22 Tax_id=10990 [Mus musculus]	P67084	15 kDa	unknown	0	2	0	0	0	2	0	0	2
51.5	TRUE	FALSE	tr Q3T1HE7 Mycosin regulatory light chain Tax_id=10990 [Mus musculus]	Q3T1HE7	20 kDa	unknown	0	5	0	0	0	1	0	0	1
51.6	TRUE	FALSE	tr P0918P6 Ribosomal protein L13 Tax_id=10990 [Mus musculus]	P0918P6	13 kDa	unknown	0	0	0	0	0	3	0	0	1
51.7	TRUE	FALSE	tr Q3T1L3 Uncharacterized protein Tax_id=10990 [Mus musculus]	Q3T1L3	13 kDa	unknown	0	0	0	0	0	3	0	0	1
51.8	TRUE	FALSE	tr Q5C769 605 ribosomal protein L14 Tax_id=10990 [Mus musculus]	P50496	23 kDa	unknown	0	1	0	1	0	1	0	0	1
51.9	TRUE	FALSE	tr Q6C9X6 405 ribosomal protein Tax_id=10990 [Mus musculus]	Q6C9X6	23 kDa	unknown	0	1	0	1	0	1	0	0	1
52	TRUE	FALSE	tr Q6C9X6 405 ribosomal protein Tax_id=10990 [Mus musculus]	Q6C9X6	23 kDa	unknown	0	1	0	1	0	1	0	0	1
52.1	TRUE	FALSE	tr D3Y369 MCg49843 Tax_id=10990 [Mus musculus]	D3Y369	17 kDa	unknown	0	3	0	0	0	2	0	0	1
52.2	TRUE	FALSE	tr P62267405 ribosomal protein S23 Tax_id=10990 [Mus musculus]	P62267405	16 kDa	unknown	0	4	0	0	0	0	0	0	0
52.3	TRUE	FALSE	tr Q3T1VH1 Uncharacterized protein Tax_id=10990 [Mus musculus]	Q3T1VH1	16 kDa	unknown	0	4	0	0	0	0	0	0	0
52.4	TRUE	FALSE	tr Q3T1VH1 Uncharacterized protein Tax_id=10990 [Mus musculus]	Q3T1VH1	16 kDa	unknown	0	4	0	0	0	0	0	0	0
52.5	TRUE	FALSE	tr Q3T1VH1 Uncharacterized protein Tax_id=10990 [Mus musculus]	Q3T1VH1	16 kDa	unknown	0	2	0	0	0	3	0	0	1
52.6	TRUE	FALSE	tr Q3T1VH1 Uncharacterized protein Tax_id=10990 [Mus musculus]	Q3T1VH1	16 kDa	unknown	0	2	0	0	0	3	0	0	1
52.7	TRUE	FALSE	tr Q3T1VH1 Uncharacterized protein Tax_id=10990 [Mus musculus]	Q3T1VH1	16 kDa	unknown	0	2	0	0	0	3	0	0	1
52.8	TRUE	FALSE	tr Q3T1VH1 Uncharacterized protein Tax_id=10990 [Mus musculus]	Q3T1VH1	16 kDa	unknown	0	2	0	0	0	3	0	0	1
52.9	TRUE	FALSE	tr Q3T1VH1 Uncharacterized protein Tax_id=10990 [Mus musculus]	Q3T1VH1	16 kDa	unknown	0	2	0	0	0	3	0	0	1
52.10	TRUE	FALSE	tr Q3T1VH1 Uncharacterized protein Tax_id=10990 [Mus musculus]	Q3T1VH1	16 kDa	unknown	0	2	0	0	0	3	0	0	1
52.11	TRUE	FALSE	tr Q3T1VH1 Uncharacterized protein Tax_id=10990 [Mus musculus]	Q3T1VH1	16 kDa	unknown	0	2	0	0	0	3	0	0	1
52.12	TRUE	FALSE	tr Q3T1VH1 Uncharacterized protein Tax_id=10990 [Mus musculus]	Q3T1VH1	16 kDa	unknown	0	2	0	0	0	3	0	0	1
52.13	TRUE	FALSE	tr Q3T1VH1 Uncharacterized protein Tax_id=10990 [Mus musculus]	Q3T1VH1	16 kDa	unknown	0	2	0	0	0	3	0	0	1

78	TRUE	FALSE	u[A1L33]DEAD (Asp-Glu-Ala-Asp) box polyepitope 5 Tax. Id=10090 [Mus musculus] A1L333	69 kDa		unknown	0	2	0	0	0	0	0	0	0
78.1	TRUE	FALSE	u[Q3V0Z8]Putative uncharacterized protein (Fragment) Tax. Id=10090 [Mus musculus] Q3V0Z8	77 kDa		unknown	0	2	0	0	0	0	0	0	0
78.2	TRUE	FALSE	u[Q5I22]2Dk3c protein (Fragment) Tax. Id=10090 [Mus musculus]	73 kDa		unknown	0	2	0	0	0	0	0	0	0
78.3	TRUE	FALSE	gpiQ61659 Probable ATP-dependent RNA helicase DDX5 Tax. Id=10090 [Mus musculus] Q61656	69 kDa		unknown	0	2	0	0	0	0	0	0	0
78.4	TRUE	FALSE	gpiQ8B1S0 DEAD (Asp-Glu-Ala-Asp) box polyepitope 3 Tax. Id=10090 [Mus musculus] Q8B1S0	69 kDa		unknown	0	2	0	0	0	0	0	0	0
79	TRUE	FALSE	u[Q10126]Elongation factor L-alpha 1 Tax. Id=10090 [Mus musculus]	50 kDa		unknown	0	0	0	0	0	1	0	0	0
79.1	TRUE	FALSE	u[Q10126]Elongation factor L-alpha 1 Tax. Id=10090 [Mus musculus]	50 kDa		unknown	0	0	0	0	0	1	0	0	0
79.2	TRUE	FALSE	gpiP10261 Elongation factor L-alpha 2 Tax. Id=10090 [Mus musculus]	50 kDa		unknown	0	0	0	0	0	1	0	0	0
79.3	TRUE	FALSE	u[Q3U3H]Elongation factor L-alpha Tax. Id=10090 [Mus musculus]	50 kDa		unknown	0	0	0	0	0	1	0	0	0
79.4	TRUE	FALSE	u[Q3U3H]Elongation factor L-alpha Tax. Id=10090 [Mus musculus]	50 kDa		unknown	0	0	0	0	0	1	0	0	0
79.5	TRUE	FALSE	u[Q3UZQ3]Uncharacterized protein Tax. Id=10090 [Mus musculus]	50 kDa		unknown	0	0	0	0	0	1	0	0	0
80	TRUE	FALSE	u[D3YTQ9]Uncharacterized protein Tax. Id=10090 [Mus musculus]	14 kDa		unknown	0	1	0	0	0	0	0	0	0
80.1	TRUE	FALSE	gpiP62843 40S ribosomal protein S15 Tax. Id=10090 [Mus musculus]	17 kDa		unknown	0	1	0	0	0	0	0	0	0
80.2	TRUE	FALSE	u[Q3UK70]Putative uncharacterized protein Tax. Id=10090 [Mus musculus]	17 kDa		unknown	0	1	0	0	0	0	0	0	0
80.3	TRUE	FALSE	u[Q3UXS6]Uncharacterized protein Tax. Id=10090 [Mus musculus]	15 kDa		unknown	0	1	0	0	0	0	0	0	0
80.4	TRUE	FALSE	refXP_003086515 PREDICTED: 40S ribosomal protein S15-like Tax. Id=10090 [Mus musculus] XP_003086515.1	17 kDa		unknown	0	1	0	0	0	0	0	0	0

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Figure 2. Analysis of the silvestrol and glycine elutions of pulldown assay using biotinylated episilvestrol (**9**) by mass spectrometry.

eIF4AI

Query	Start	End	Observed	Mr(expt)	Mr(calc)	ppm	M	Score	Expect	Rank	U	Peptide		
625	46	61	914.4726	1826.9306	1826.9315	-0.47	1	40	0.00018	1	U	R.GIYAYGFEKPSAIQQ.R.A		
626	46	61	914.4736	1826.9326	1826.9315	0.63	1	45	6.00E-05	1	U	R.GIYAYGFEKPSAIQQ.R.A		
627	46	61	609.9849	1826.9329	1826.9315	0.75	1	36	0.00056	1	U	R.GIYAYGFEKPSAIQQ.R.A		
628	46	61	609.985	1826.9332	1826.9315	0.91	1	34	0.0029	1	U	R.GIYAYGFEKPSAIQQ.R.A		
56	62	68	407.7463	813.478	813.4782	-0.22	0	4	0.39	1	U	R.AILPCIK.G		
57	62	68	407.7466	813.4786	813.4782	0.52	0	3	0.5	1	U	R.AILPCIK.G		
548	69	82	697.8491	1393.6836	1393.6838	-0.082	0	115	1.40E-10	1	U	K.GYDVIAQAQSGTGK.T		
549	69	82	697.8494	1393.6842	1393.6838	0.35	0	92	7.70E-09	1	U	K.GYDVIAQAQSGTGK.T		
361	100	110	570.8405	1139.6664	1139.6662	0.18	0	31	0.014	1	U	K.ATQALVLAPTR.E		
362	100	110	570.8405	1139.6664	1139.6662	0.18	0	36	0.0044	1	U	K.ATQALVLAPTR.E		
170	111	118	479.2716	956.5286	956.5291	-0.44	0	29	0.035	1	U	R.ELAQQIQK.V		
171	111	118	479.2719	956.5292	956.5291	0.19	0	35	0.0092	1	U	R.ELAQQIQK.V		
606	147	161	540.2956	1617.865	1617.8661	-0.69	0	45	0.0016	1	U	K.LOMEAPHIVGTPGR.V		
607	147	161	540.2959	1617.8659	1617.8661	-0.13	0	31	0.014	1	U	K.LOMEAPHIVGTPGR.V		
608	147	161	809.9404	1617.8662	1617.8661	0.098	0	41	0.00035	1	U	K.LOMEAPHIVGTPGR.V		
609	147	161	809.9405	1617.8664	1617.8661	0.22	0	61	4.00E-06	1	U	K.LOMEAPHIVGTPGR.V		
110	162	168	447.7287	893.4428	893.4429	-0.077	0	25	0.015	1	U	R.VFDMLNR.R		
111	162	168	447.7288	893.443	893.4429	0.15	0	26	0.018	1	U	R.VFDMLNR.R		
260	162	169	525.7791	1049.5436	1049.544	-0.36	1	11	0.53	1	U	R.VFDMLNRR.Y		
584	178	190	778.3596	1554.7046	1554.7058	-0.74	0	61	6.00E-06	1	U	K.MFVLDEADEMLSR.G		
585	178	190	778.3598	1554.705	1554.7058	-0.48	0	69	2.40E-06	1	U	K.MFVLDEADEMLSR.G		
579	191	202	751.3873	1500.76	1500.7613	-0.82	1	64	2.60E-06	1	U	R.GFKDOIYDIFOK.L		
580	191	202	751.3879	1500.7612	1500.7613	-0.019	1	59	1.70E-05	1	U	R.GFKDOIYDIFOK.L		
386	194	202	585.2953	1168.576	1168.5764	-0.33	0	27	0.011	1	U	K.DQIYAIIFOK.L		
387	194	202	585.2955	1168.5764	1168.5764	0.015	0	35	0.0078	1	U	K.DQIYAIIFOK.L		
145	227	233	467.7496	933.4846	933.4854	-0.85	1	11	3.5	6	U	K.FMRDPIR.I		
146	227	233	467.75	933.4854	933.4854	0.0054	1	9	5.7	5	U	K.FMRDPIR.I		
400	238	247	594.3345	1186.6544	1186.6557	-1.07	1	29	0.061	1	U	K.KEELTLEGIR.Q		
401	238	247	396.559	1186.6552	1186.6557	-0.46	1	22	0.27	2	U	K.KEELTLEGIR.Q		
402	238	247	594.335	1186.6554	1186.6557	-0.23	1	43	0.0023	1	U	K.KEELTLEGIR.Q		

403	238	247	396.5593	1186.6561	1186.6557	0.3	1	22	0.3	3	U	K.KEELTLEGIR.Q
267	239	247	530.2869	1058.5592	1058.5608	-1.43	0	32	0.0085	1	U	K.EELTLEGIR.Q
268	239	247	530.288	1058.5614	1058.5608	0.65	0	35	0.0059	1	U	K.EELTLEGIR.Q
280	248	255	534.7767	1067.5388	1067.54	-1.06	0	12	0.26	1	U	R.QFYINVER.E
225	284	291	509.7816	1017.5486	1017.5495	-0.82	1	22	0.089	1	U	R.KVDWLTEK.M
226	284	291	509.7821	1017.5496	1017.5495	0.16	1	19	0.093	1	U	R.KVDWLTEK.M
108	285	291	445.7346	889.4546	889.4545	0.13	0	9	0.22	1	U	K.VDWLTEK.M
109	285	291	445.7347	889.4548	889.4545	0.36	0	18	0.18	1	U	K.VDWLTEK.M
589	296	309	791.3364	1580.6582	1580.6599	-1.07	0	55	7.20E-06	1	U	R.DFTVSAMHGDMQ.K.E
590	296	309	527.8936	1580.659	1580.6599	-0.61	0	3	0.62	1	U	R.DFTVSAMHGDMQ.K.E
591	296	309	527.8939	1580.6599	1580.6599	-0.045	0	9	0.15	1	U	R.DFTVSAMHGDMQ.K.E
592	296	309	791.3373	1580.66	1580.6599	0.066	0	56	5.00E-06	1	U	R.DFTVSAMHGDMQ.K.E
326	325	334	557.8445	1113.6744	1113.6758	-1.17	0	65	6.00E-06	1	U	R.VLIITDLLAR.G
327	325	334	557.8448	1113.675	1113.6758	-0.63	0	39	0.00044	1	U	R.VLIITDLLAR.G
69	354	359	416.2089	830.4032	830.4035	-0.27	0	27	0.044	1	U	R.ENYIHR.I
70	354	359	416.209	830.4034	830.4035	-0.03	0	26	0.045	1	U	R.ENYIHR.I
597	369	382	530.6152	1588.8238	1588.8243	-0.31	2	57	4.00E-05	1	U	R.KGVAINMVTEEDKR.T
598	369	382	530.6153	1588.8241	1588.8243	-0.12	2	48	0.00018	1	U	R.KGVAINMVTEEDKR.T
599	369	382	795.4195	1588.8244	1588.8243	0.12	2	50	2.60E-05	1	U	R.KGVAINMVTEEDKR.T
566	370	382	731.3718	1460.729	1460.7293	-0.18	1	44	7.90E-05	1	U	K.GVAINMVTEEDKR.T
567	370	382	731.3722	1460.7298	1460.7293	0.37	1	33	0.0021	1	U	K.GVAINMVTEEDKR.T

eIF4A II

Query	Start	End	Observed	Mr(expt)	Mr(calc)	ppm	M	Score	Expect	Rank	U	Peptide
625	47	62	914.4726	1826.9306	1826.9315	-0.47	1	40	0.00018	1	U	R.GIYAYGFEKPSAIQQ.R.A
626	47	62	914.4736	1826.9326	1826.9315	0.63	1	45	6.00E-05	1	U	R.GIYAYGFEKPSAIQQ.R.A
627	47	62	609.9849	1826.9329	1826.9315	0.75	1	36	0.00056	1	U	R.GIYAYGFEKPSAIQQ.R.A
628	47	62	609.985	1826.9332	1826.9315	0.91	1	34	0.0029	1	U	R.GIYAYGFEKPSAIQQ.R.A
56	63	69	407.7463	813.478	813.4782	-0.22	0	4	0.39	1	U	R.AIIPCIK.G
57	63	69	407.7466	813.4786	813.4782	0.52	0	3	0.5	1	U	R.AIIPCIK.G
548	70	83	697.8491	1393.6836	1393.6838	-0.082	0	115	1.40E-10	1	U	K.GYDVIAQAQSGTGK.T
549	70	83	697.8494	1393.6842	1393.6838	0.35	0	92	7.70E-09	1	U	K.GYDVIAQAQSGTGK.T
170	112	119	479.2716	956.5286	956.5291	-0.44	0	29	0.035	1	U	R.ELAQQIQK.V
171	112	119	479.2719	956.5292	956.5291	0.19	0	35	0.0092	1	U	R.ELAQQIQK.V
110	163	169	447.7287	893.4428	893.4429	-0.077	0	25	0.015	1	U	R.VFDMLNR.R
111	163	169	447.7288	893.443	893.4429	0.15	0	26	0.018	1	U	R.VFDMLNR.R
260	163	170	525.7791	1049.5436	1049.544	-0.36	1	11	0.53	1	U	R.VFDMLNR.R.Y
584	179	191	778.3596	1554.7046	1554.7058	-0.74	0	61	6.00E-06	1	U	K.MFVLDEADEMLSR.G
585	179	191	778.3598	1554.705	1554.7058	-0.48	0	69	2.40E-06	1	U	K.MFVLDEADEMLSR.G
280	249	256	534.7767	1067.5388	1067.54	-1.06	0	12	0.26	1	U	K.QFYINVER.E
225	285	292	509.7816	1017.5486	1017.5495	-0.82	1	22	0.089	1	U	R.KVDWLTEK.M
226	285	292	509.7821	1017.5496	1017.5495	0.16	1	19	0.093	1	U	R.KVDWLTEK.M

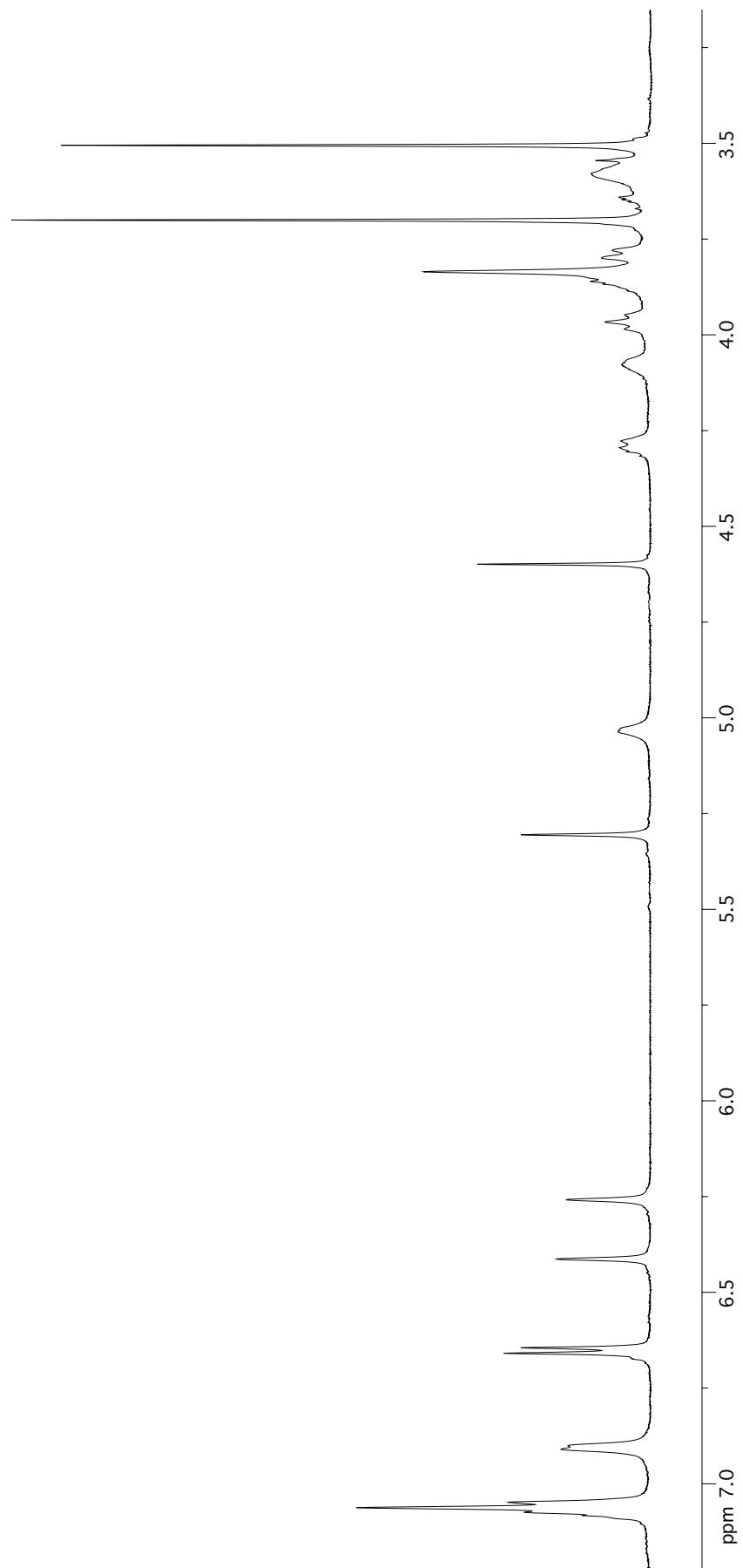
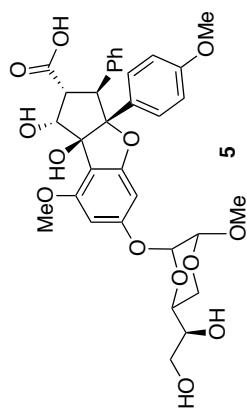
108	286	292	445.7346	889.4546	889.4545	0.13	0	9	0.22	1	U	K.VDWLTEK.M
109	286	292	445.7347	889.4548	889.4545	0.36	0	18	0.18	1	U	K.VDWLTEK.M
326	326	335	557.8445	1113.6744	1113.6758	-1.17	0	65	6.00E-06	1	U	R.VLITTDLLAR.G
327	326	335	557.8448	1113.675	1113.6758	-0.63	0	39	0.00044	1	U	R.VLITTDLLAR.G
69	355	360	416.2089	830.4032	830.4035	-0.27	0	27	0.044	1	U	R.ENYIHR.I
70	355	360	416.209	830.4034	830.4035	-0.03	0	26	0.045	1	U	R.ENYIHR.I

Figure 3. eIF4A isoform comparison by mass spectrometry. Peptides in *italics* are eIF4AI specific.

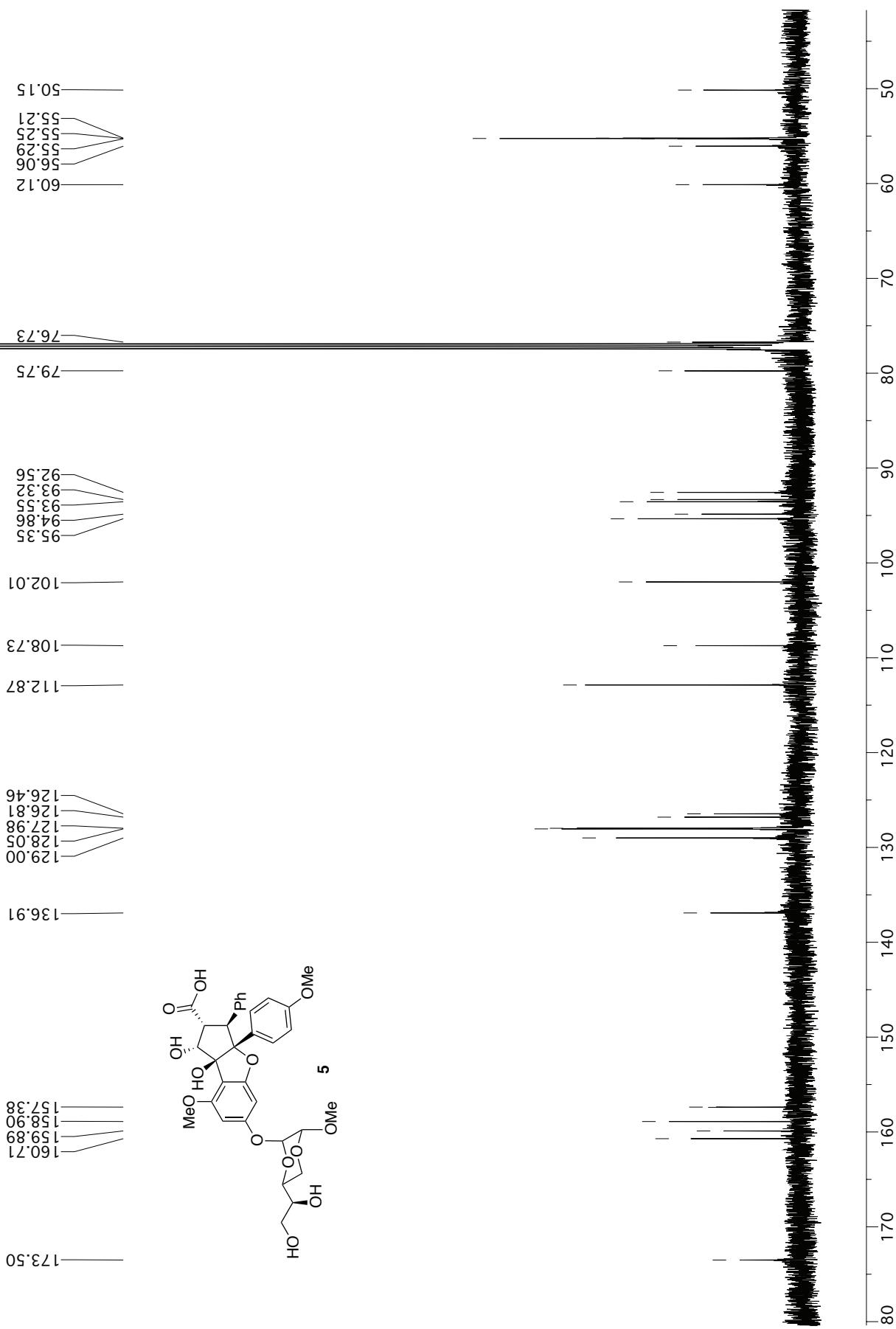
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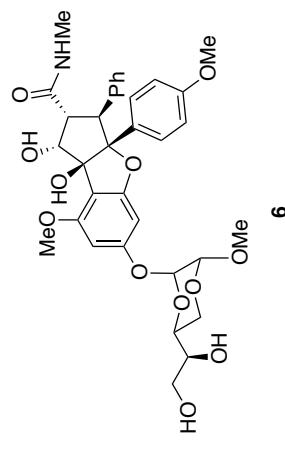
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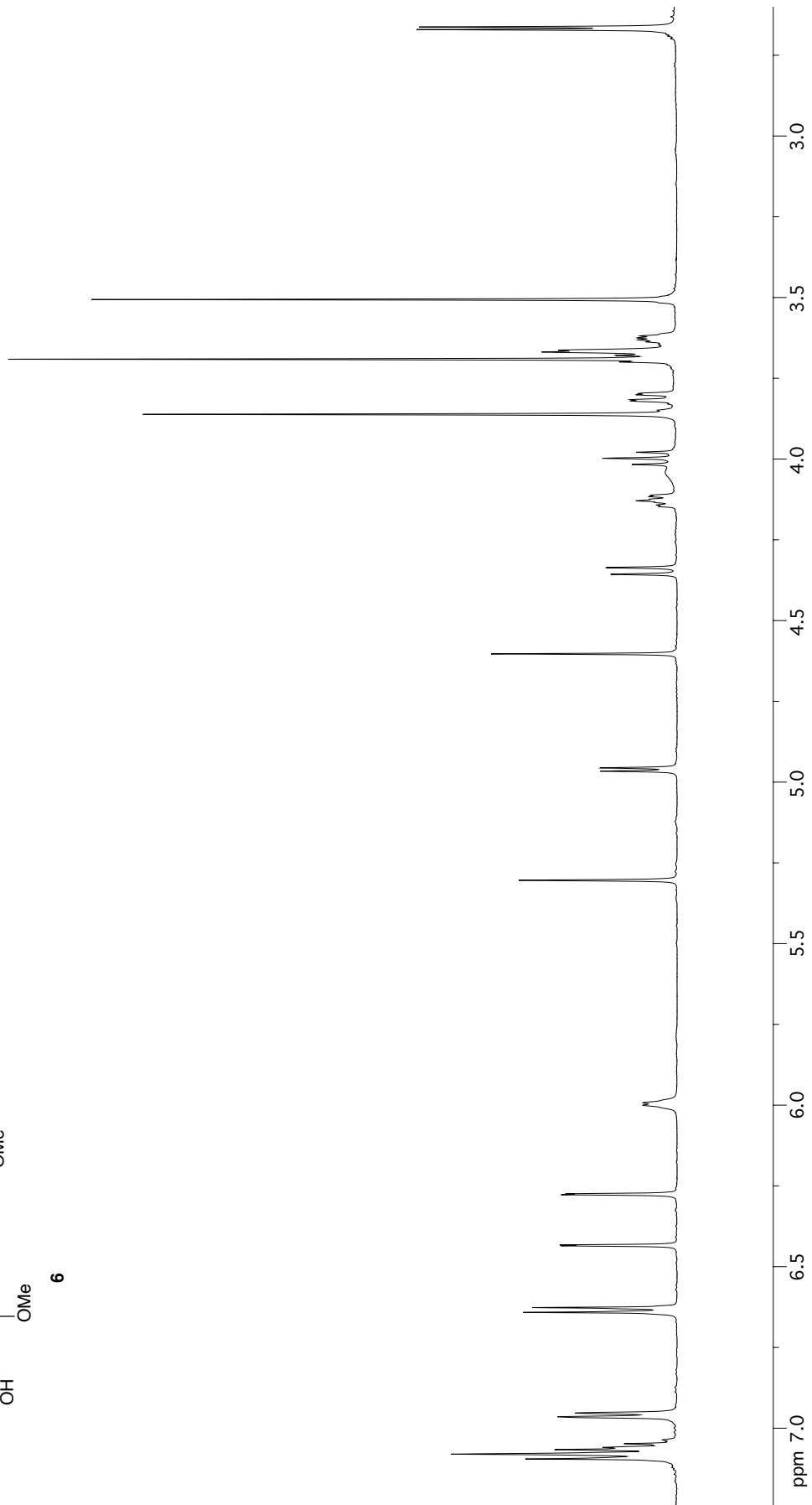
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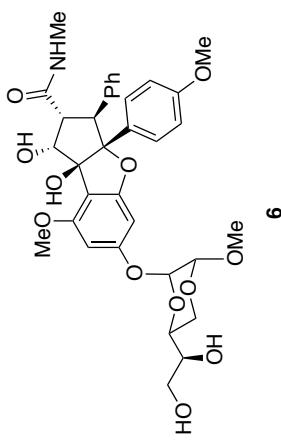
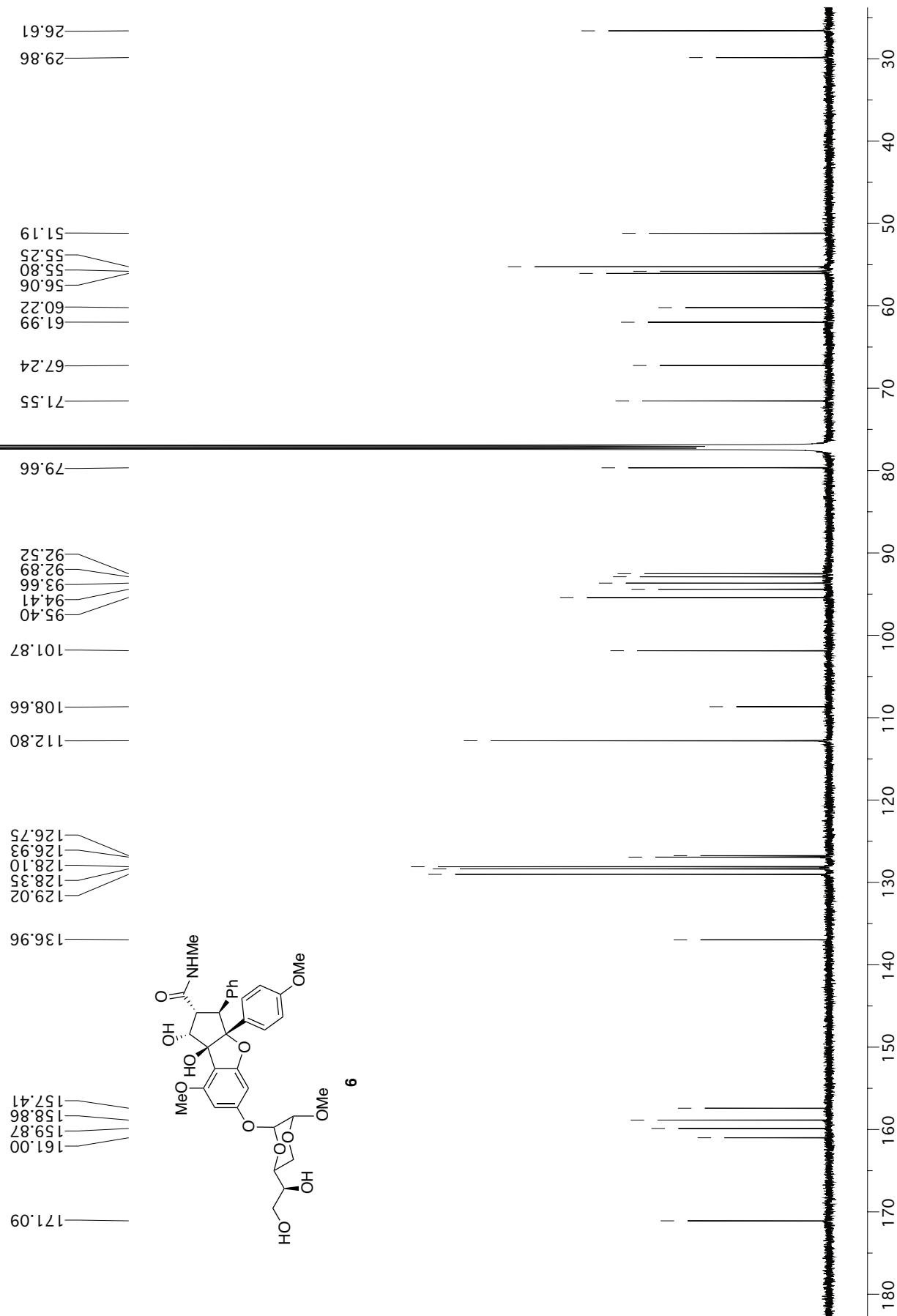
¹H NMR (600 MHz, CDCl₃)



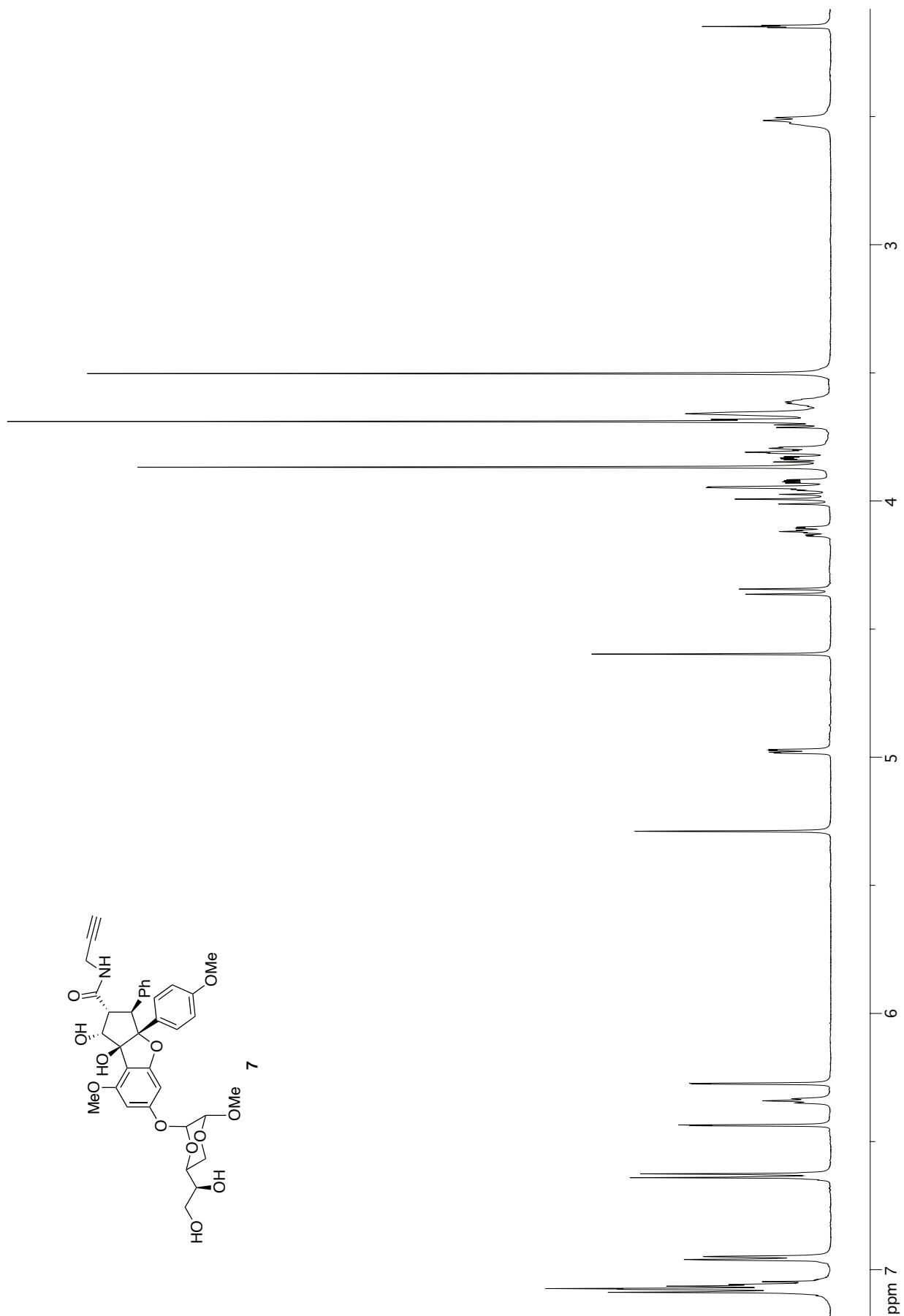
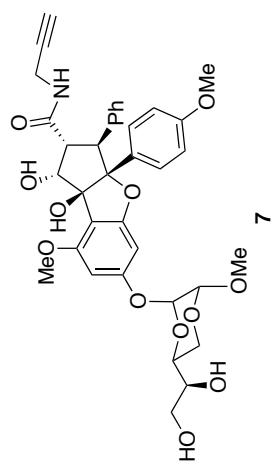
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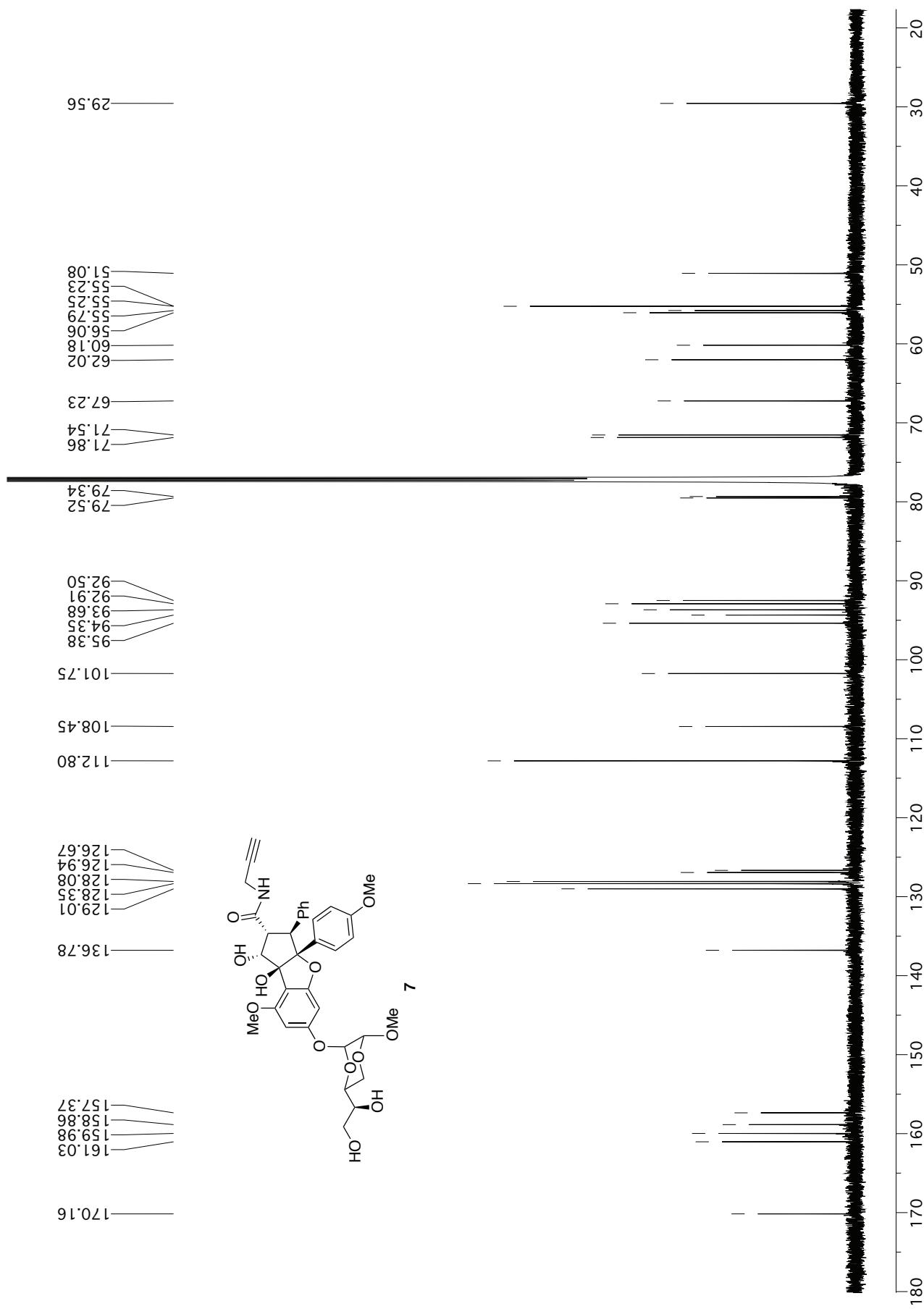


¹³C NMR (150 MHz, CDCl₃)

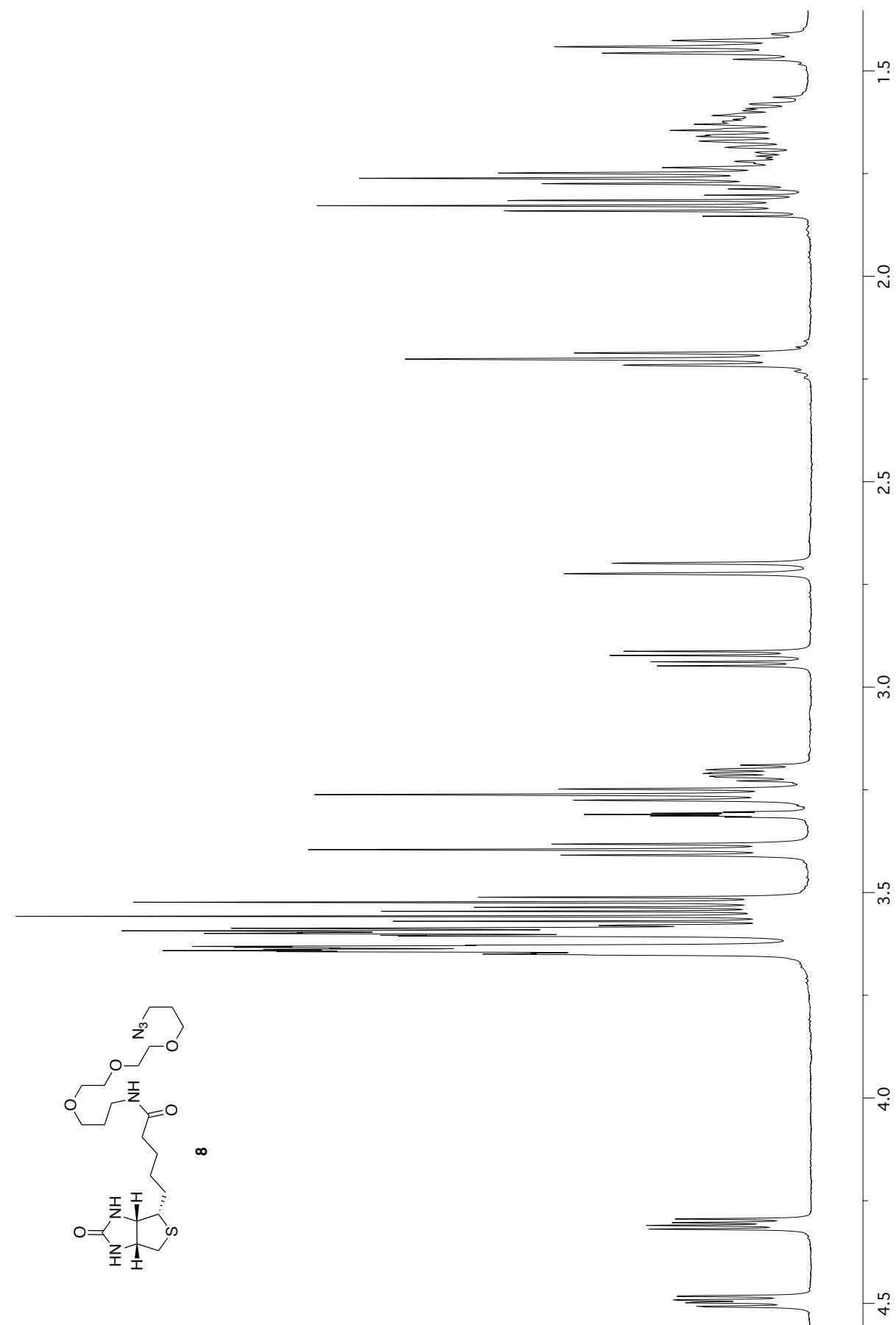
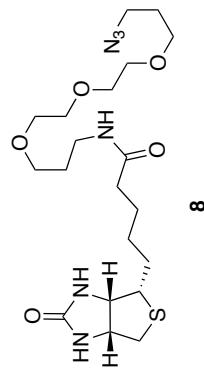


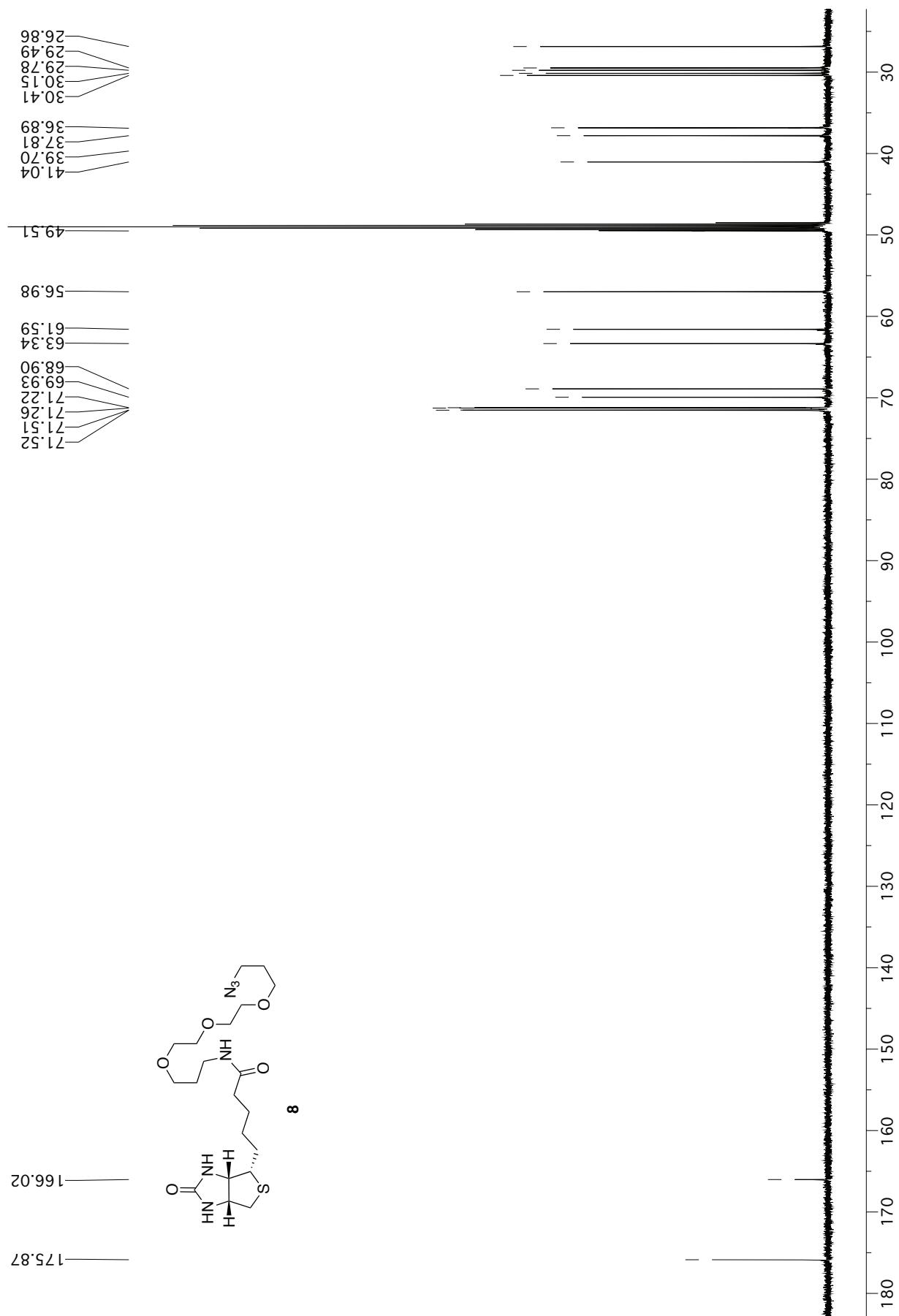
¹H NMR (600 MHz, CDCl₃)



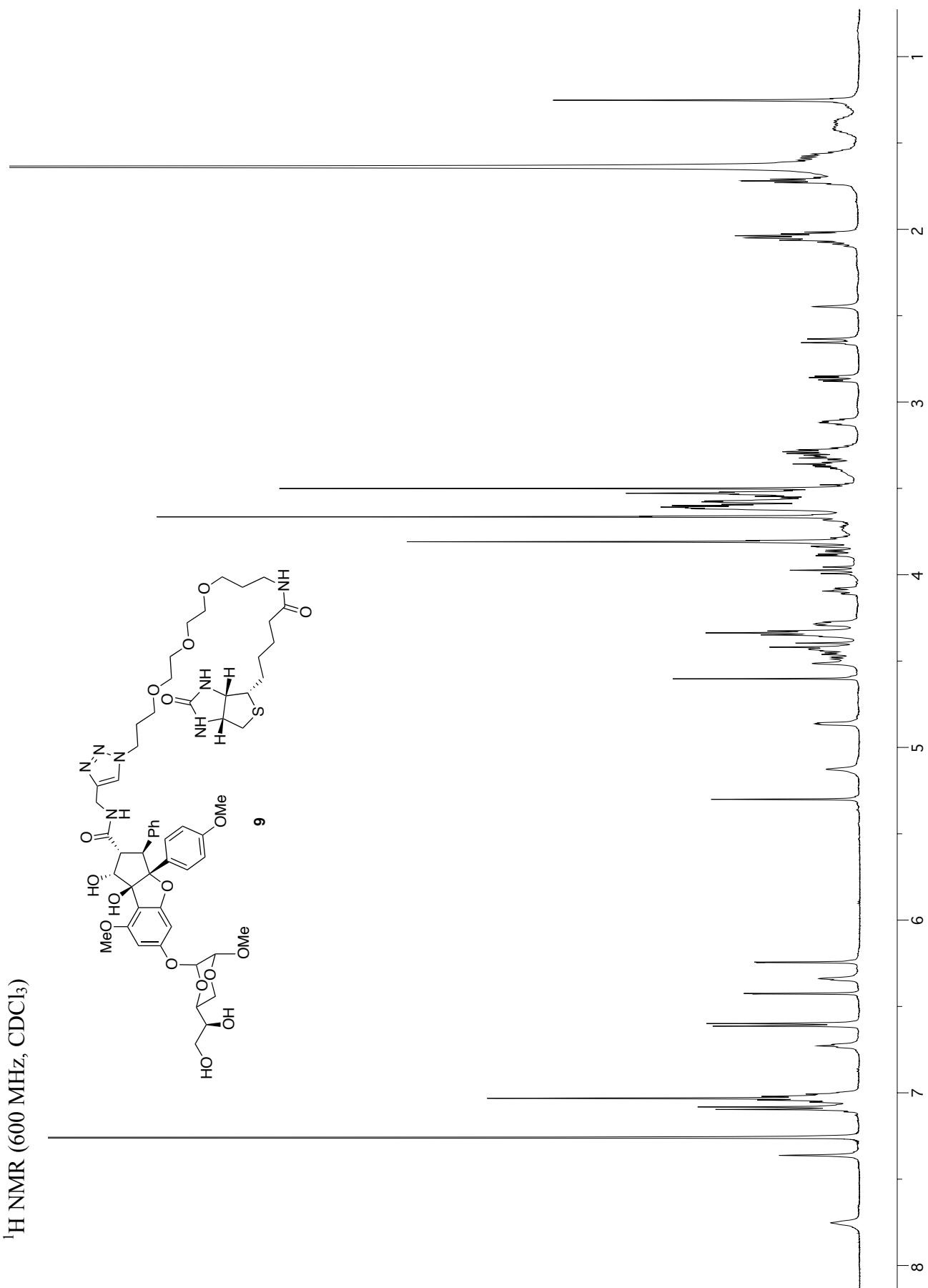
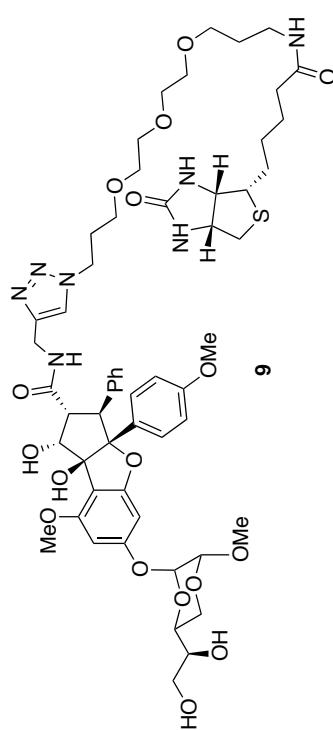


¹H NMR (500 MHz, CDCl₃)





¹H NMR (600 MHz, CDCl₃)



¹³C NMR (150 MHz, CDCl₃)

