Supporting Information

Chemical Genetic Control of Protein Levels: Selective *in vivo* Targeted Degradation

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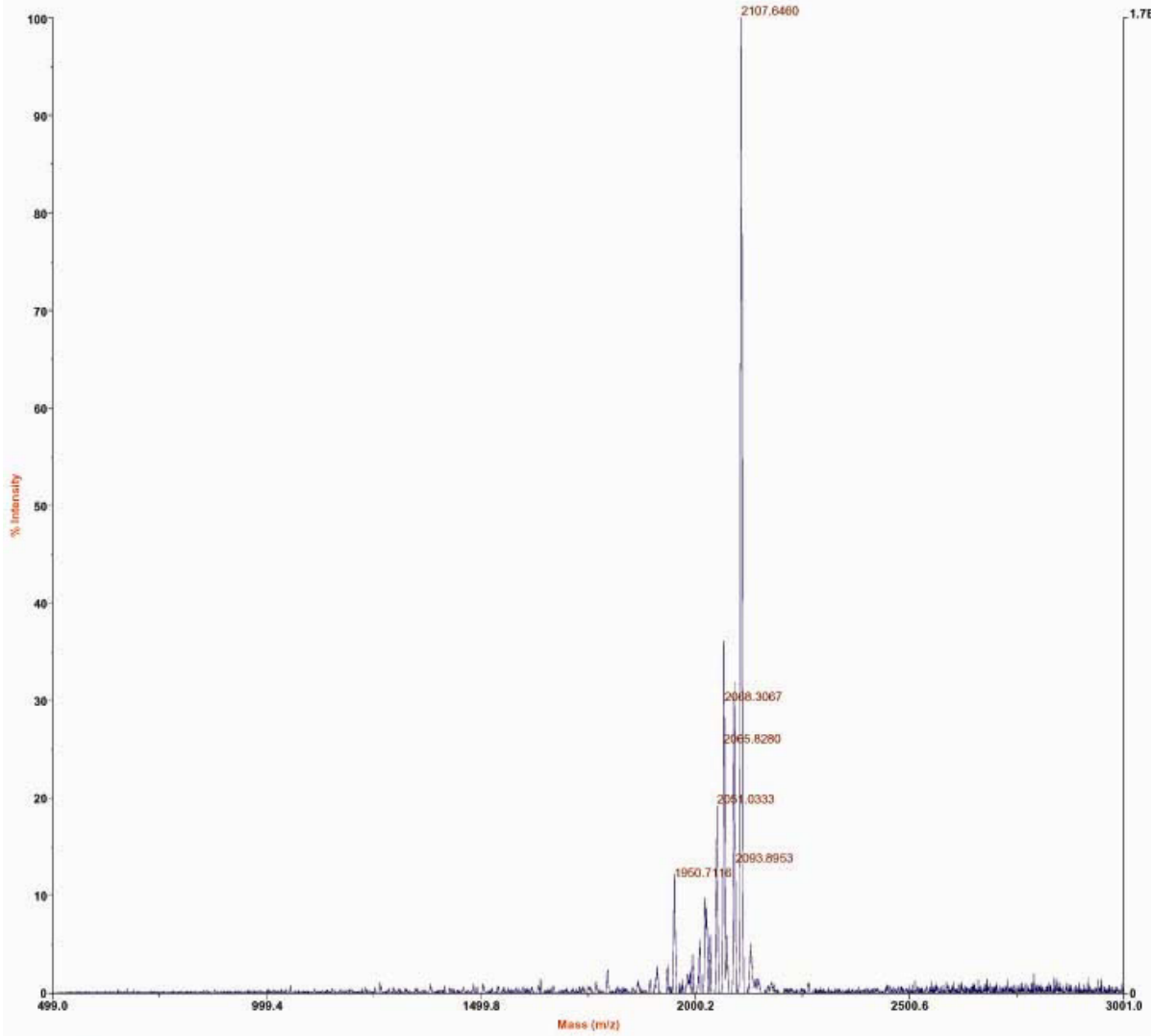
Purification of HIF-polyarginine Peptide (HIF1)

HIF1 (H_2N-$(CH_2)_5$CO-ALAPYIP-(*D*-Arg)$_8$$NH_2$) was purified by RP-HPLC (Rainin Dynamax System) comprising two solvent delivery pumps (Model SD200) and variable wavelength detector (model UV-1) set at 214nm. The column used was an YMC-Pack

ODS-AM, 250 x 20 mm, 5μm particle size and 120Å pore size (Waters, Milford, MA) coupled to a guard column ODSA (10 x 10mm) with the same stationary phase specifications. The separation was carried out with a linear gradient of solvent A (H_2O/CH_3CN 98:2 + 0.06% of TFA) into solvent B (CH_3CN/H_2O 80:20 + 0.05% of TFA): 15% of B to 35% of B over 85min, 35% B to 98% B over 15 min, run at 5.0ml/min. HIF1 was eluted at 69 min. MALDI-TOF analysis of peptide samples were performed in a Voyager-DE- PRO 6268 (Applied Biosystems) using a-cyano-4-hydroxycinnamic acid matrices. Analytical HPLC traces of purified samples was carried out in a Waters Separation Module 2795 coupled to a Waters 2795 Photodiode Array Detector (set at 214nm) and to a Micromass ZQ 4000 Electrospray Mass detector (cone voltage = +120V). A linear gradient of Solvent A (composition as described for preparative separations) into Solvent B, 2% B to 37.5% B over 63min, was run at 0.2ml/min. The analytical column used was an XTerra MS C18 (4.6mm x 50mm, 2.5μm particle size, 80Å pore size).

Applied Biosystems Voyager System 6268

Voyager Spec #1=>NF1.0=>AdvBC(32,0.5,0.1)[BP = 2107.7, 16505]



Mode of operation:	Reflector
Extraction mode:	Delayed
Polarity:	Positive
Acquisition control:	Manual
Accelerating voltage:	20000 V
Grid voltage:	94%
Mirror voltage ratio:	1.12
Guide wire 0:	0.0025%
Extraction delay time:	185 nsec
Acquisition mass range:	500 -- 3000 Da
Number of laser shots:	200/spectrum
Laser intensity:	2227
Laser Rep Rate:	20.0 Hz
Calibration type:	Default
Calibration matrix:	a-Cyano-4-hydroxycinnamic acic
Low mass gate:	500 Da
Timed ion selector:	Off
Digitizer start time:	22.699
Bin size:	0.5 nsec
Number of data points:	65433
Vertical scale 0:	500 mV
Vertical offset:	1.5%
Input bandwidth 0:	500 MHz
Sample well:	15
Plate ID:	100 WELL PLATE
Serial number:	6268
Instrument name:	Voyager-DE PRO
Plate type filename:	C:\VOYAGER\100 well plate.plt
Lab name:	Yale University
Absolute x-position:	22624.3
Absolute y-position:	41422
Relative x-position:	716.77
Relative y-posititon:	-805.514
Shots in spectrum:	200
Source pressure:	3.218e-007
Mirror pressure:	6.037e-008
TC2 pressure:	0.001
TIS gate width:	8
TIS flight length:	690.5

Acquired: 12:14:00, March 06, 2003

Printed: 16:31, September 26, 2003

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Figure 1: MALDI-TOF spectrum of purified HIF 1 sample used on the synthesis of (**3**).

Analytical conditions are described above.

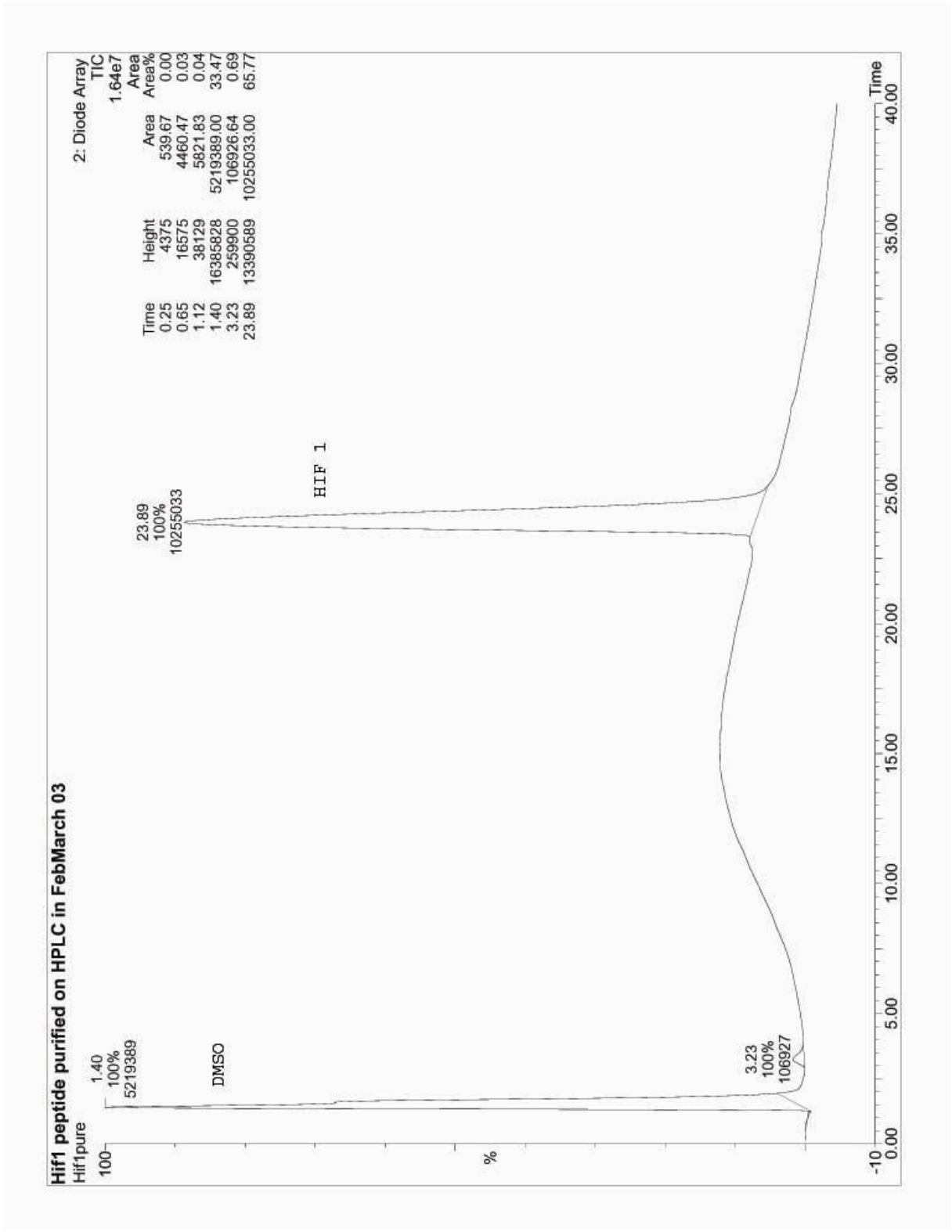

Figure 2: HPLC chromatogram (UV trace) of purified HIF 1 used in the synthesis of Protac-2. Analytical conditions are described above.

Preparation of PROTAC-4 (3).

AP21998 (**1**) was synthesized as previously described[21,22], as a 1:1 mixture of diastereomers at C9. To a solution of 15 mg (0.021mmol) of 1 in 1mL DMF was added 6.8mg (0.023mmol) EDCI, 6.4mg (0.053mmol) DMAP, and 4.6mg (0.021mmol) H_2N-$(CH_2)_5CO_2Bn$. The reaction was stirred for 24h, and filtered through Celite filter agent. Solvent was removed under vacuum, and the crude product (16mg) was redissolved in 2mL ethanol. To this solution was added 20mg 10% Pd/C, the reaction was placed under an atmosphere of hydrogen (balloon) and stirred for 1h. The reaction was filtered through Celite, after which the solvent was removed *in vacuo* to yield 12mg of crude product (**2**). This material was carried on without further purification.

Crude **2** (12mg, 0.014mmol) was added to a 5mL round bottomed flask along with 3mg (0.0014mmol) HIF1 H_2N-$(CH_2)_5$CO-ALAPYIP-(*D*-Arg)$_8NH_2$ (W.M. Keck Foundation Biotechnology Resource Laboratory), 1mg PyBrOP, 1µL diisopropylethylamine, and 0.6mL dry DMF. The reaction mixture was stirred for 18 hours, after which solvent was evaporated under a high vacuum.

Crude mixture of **3** was purified by RP-HPLC (for HPLC system and column specifications see the purification procedure for HIF 1). A linear gradient of Solvent A into B (same solvents composition as described for HIF 1) consisting of 2% B to 37% B over 63 min, 37% B to 75% B over 37 min, 75% B to 98% B over 10 min, 98% B for 20

min, was run at 5.0 mL/min. 3 was eluted at 95 min. MALDI-TOF analyses of purified samples were performed in a Voyager-DE- PRO 6268 (Applied Biosystems) using a-cyano-4-hydroxycinnamic acid matrices. Purified samples were analyzed by LC-MS (see HIF 1 for equipment, column specifications and solvent composition and analytical parameters) using a linear gradient of B into A: 2% B to 100% B over 40 min, 100% B for 20 min, run at 0.2 mL/min.

Compound 1. ^{1}H NMR ($CDCl_3$, 400 MHz) (Mixture of rotamers and diastereomers) **1(S)** 7.19 (t, J=7.9Hz, 1H), 6.67-6.96 (m, 6H) 6.24 (s, 2H), 5.48-5.50 (m, 2H) 4.60-4.74 (m, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 3.78 (s, 3H), 3.61-3.93 (m, 2H), 3.55 (s, 6H), 2.85-2.93 (m, 1H), 2.56-2.68 (m, 2H), 1.98-2.36 (m, 4H), 1.63-1.77 (m, 4H), 1.26-1.46 (m, 2H), 0.90 (t, J-7.3Hz, 3H). **1(R)** 7.25 (t, J=7.8 Hz), 6.70-6.90 (m, 6H), 6.45 (s, 2H), 5.66-5.70 (m, 1H), 5.53 (d, J=4.5Hz, 1H), 4.59-4.72 (m, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 3.84 (s, 3H), 3.83 (s, 6H), 3.47-3.79 (m, 2H), 3.23-3.32 (m, 1H), 2.52-2.71 (m, 2H), 2.00-2.31 (m, 4H), 1.52-1.75 (m, 4H), 1.28-1.46 (m 2H), 0.83 (t, J=7.3Hz, 3H); LRMS (ES) (M + H)+ calc 694.32, meas. 694.7.

Structural assignment of **1** based on original synthesis: Yang, W., et. al. *J. Med. Chem.* **2000**, *43,* 1135-1142.

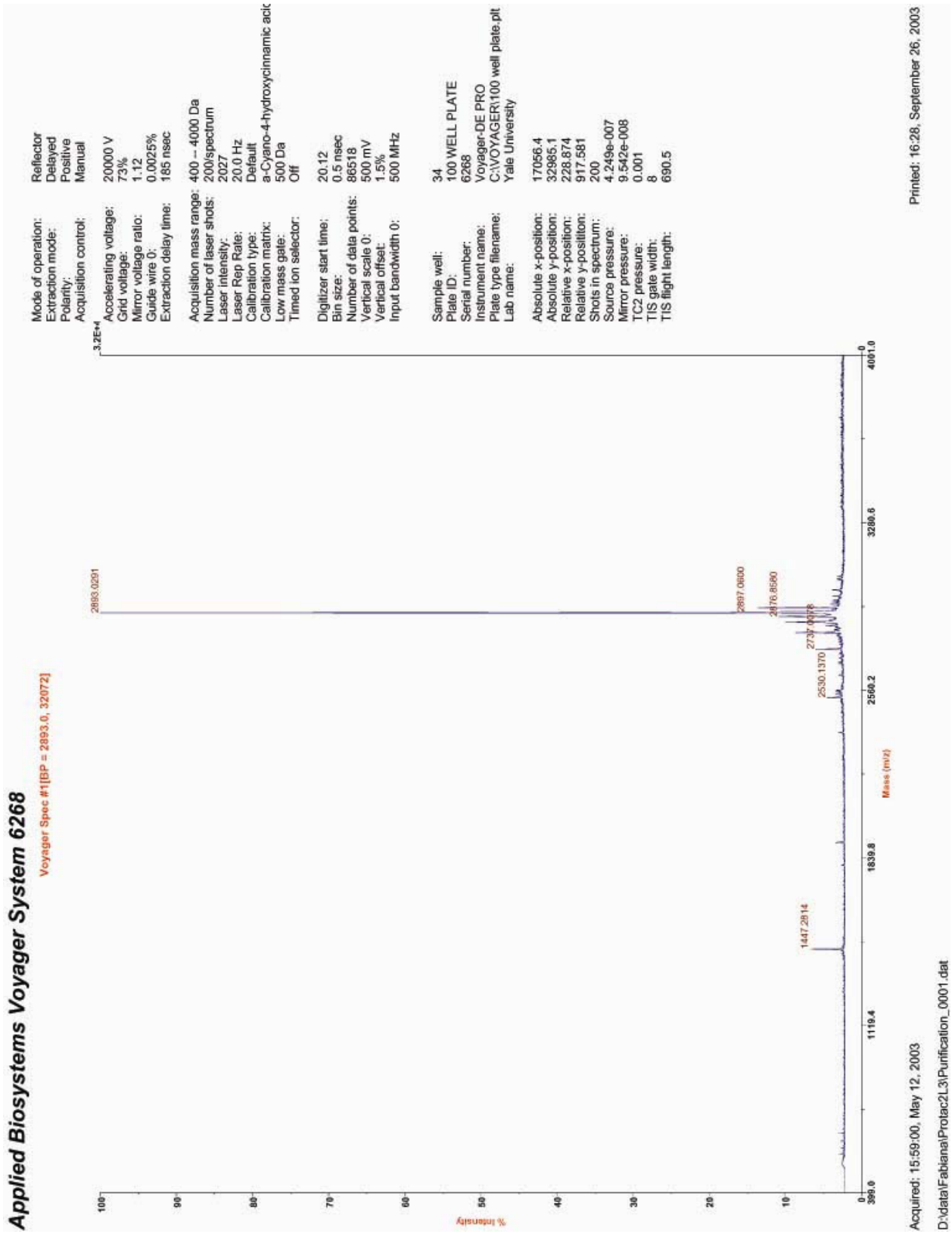

Figure 3: MALDI-TOF spectrum of purified **3**. Analytical conditions described above.

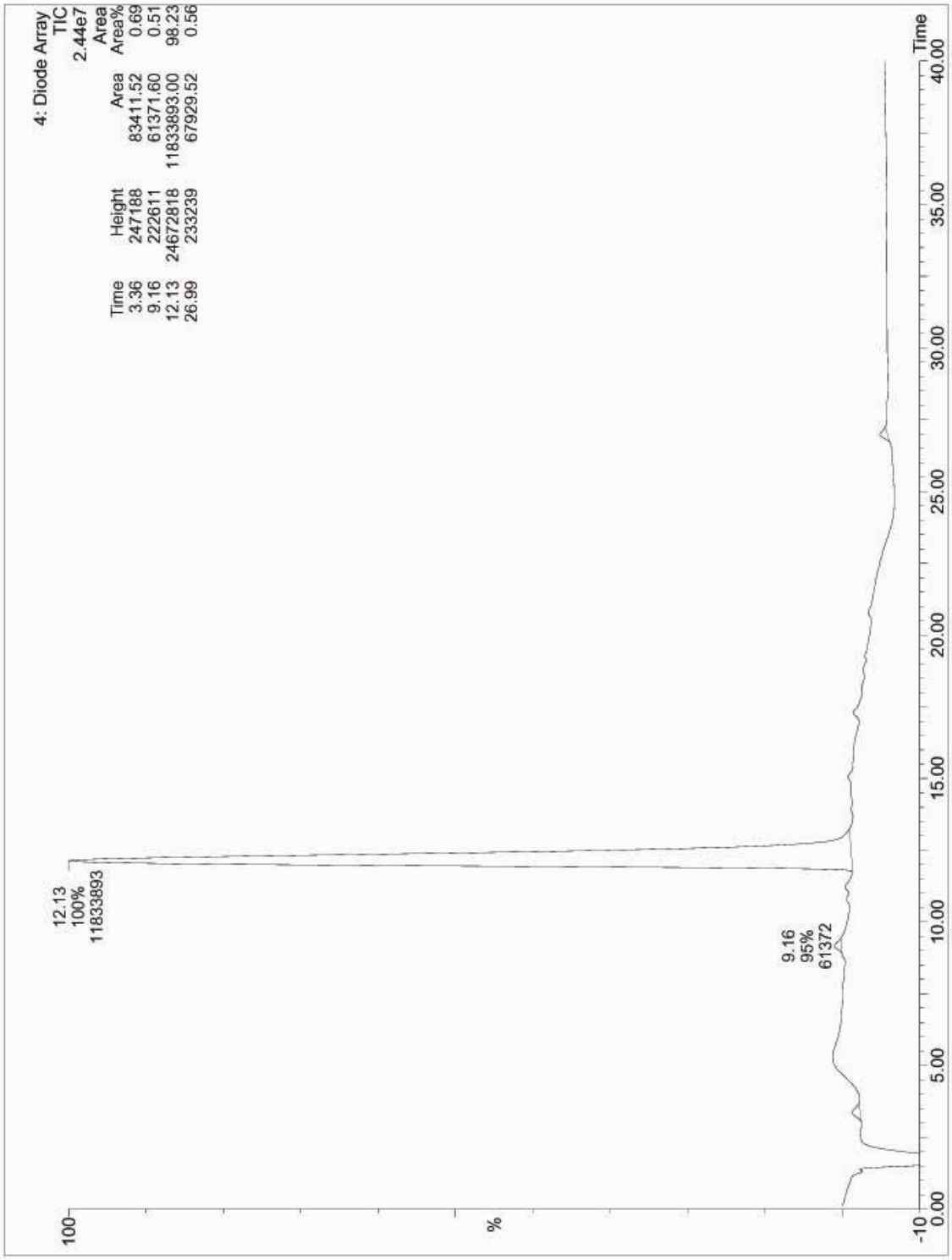

Figure 4: HPLC chromatogram (UV trace) of purified **3**. Analytical conditions described above.

Preparation of PROTAC-5 (5).

Compound **4** was synthesized as previously described[33]. To a solution of 1.2mg (0.003mmol) 4 in 1mL DMF was added 2mg H_2N-$(CH_2)_5$CO-ALAPYIP-(*D*-Arg)$_8NH_2$, 0.3mg (0.014mmol) EDCI, and 0.2mg (0.015mmol) DMAP. The reaction mixture was stirred for 24h, after which solvent was evaporated under high vacuum. The product was purified by HPLC to yield 1.3mg **5**.

The purification procedure was carried out using same equipment, column and Solvents A and B composition as described for HIF 1 and PROTAC-4 (**3**). A linear gradient was done by adding Solvent B into Solvent A: 40%B to 70%B over 100min, 70%B to 98%B over 20min, run at 5.0mL/min. Compound 5 eluted at 30min. Purified samples were analyzed by MALDI-TOF using the same parameters as described earlier. Purified samples of 5 were analyzed by LC-MS using a linear gradient consisting of 2%B to 40%B over 40min. Analytical conditions, equipment and parameters were the same used for HIF 1 and **3**.

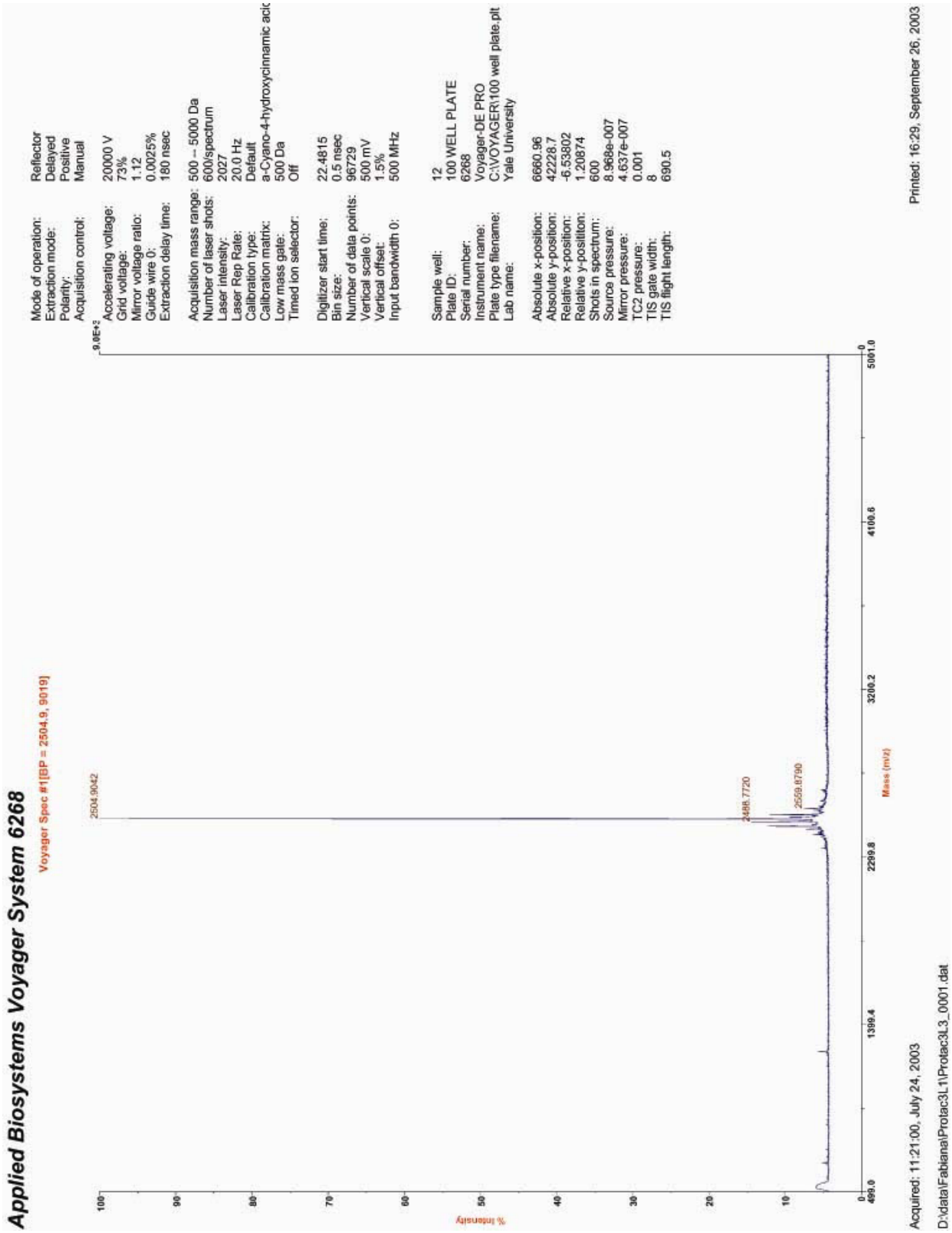

Figure 5: MALDI-TOF spectrum of purified **5**. Analytical conditions are described above.

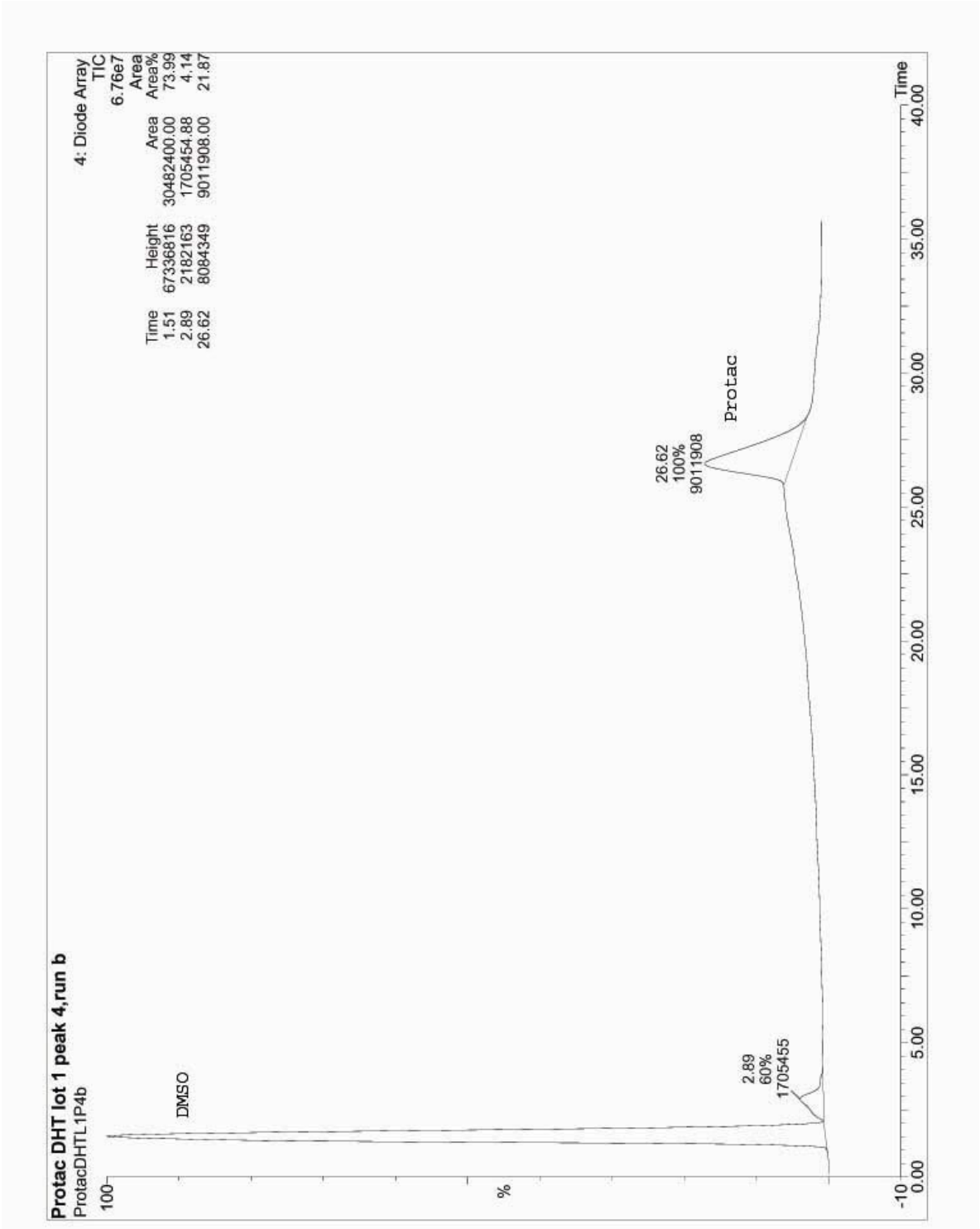

Figure 6: HPLC chromatogram (UV trace) of purified **5**. Analytical conditions are described above.