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Supplementary Information for:

N^{ε}-methanesulfonyl-lysine as a non-hydrolyzable functional surrogate for N^{ε}-acetyl-lysine

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Experimental

Synthesis of N$^{\alpha}$-Fmoc-N$^{\varepsilon}$-methanesulfonyl-lysine. (a) To a stirred solution of N$^{\alpha}$-Boc-lysine-OMe • CH_3COOH (Bachem) (260 mg, 1 mmole) in dichloromethane (10 mL) was added dropwise at 0 °C a 0.4 M solution of N-methylmorpholine (NMM) in dichloromethane (10 mL), followed by methanesulfonyl chloride (117 μL, 1.5 mmole). After the addition was complete, the reaction mixture was stirred at room temperature for 3 h before the addition of a 6.0 M aqueous NaOH solution (200 μL in 20 μL portions) at 0 °C to destroy excess methanesulfonyl chloride. The volatiles of the resulting mixture were removed under reduced pressure, affording an oily residue. (b) To the above-obtained oily residue was added dropwise at 0 °C a solution of LiOH (211 mg, 1 mmole) in dd H_2O (4.2 mL) and methanol (12 mL) while stirring. The reaction mixture was stirred at 4 °C overnight, neutralized at 0 °C with trifluoroacetic acid, and concentrated under reduced pressure, affording an oily residue (~5 mL). (c) To the above-obtained residue was added dropwise at 0 °C trifluoroacetic acid (5 mL) while stirring, and the reaction mixture was stirred at room temperature for 3 h before concentrated under reduced pressure, affording an oily residue. (d) To the above-obtained oily residue was added dd H_2O (5 mL), and the mixture was neutralized at 0 °C while stirring with a 10% (w/v) aqueous Na_2CO_3 solution. To the obtained solution was added another portion of the 10% (w/v) Na_2CO_3 solution (5 mL). A solution of Fmoc-OSu (675 mg, 2 mmole) in 1,4-dioxane (5 mL) was then added dropwise at room temperature. After the addition was complete, the reaction mixture was stirred at room temperature for 5 h before ddH_2O (50 mL) was added. The excess Fmoc-OSu was extracted away with diethyl ether (2x100 mL). The aqueous layer was acidified with a 6.0 M aqueous HCl solution to pH ~1 at 0 °C before being extracted with ethyl acetate (3x100 mL).

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The organics were combined, dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure, affording an oily residue from which the desired product was isolated by silica gel column chromatography (eluting with ethyl acetate → methanol/dichloromethane (1/10 → 1/3)) as a white solid: (200 mg, 45% after four steps): ^{1}H NMR (300 MHz, CD_3OD): δ 7.69-7.17 (m, 8H, H_{arom}), 4.30-3.90 (m, 4H, Fluorenyl H_9, CH_2O, and H_{alpha}), 2.93 (d, 2H, J=6.9 Hz, CH_2NH), 2.78 (s, 3H, CH_3), 1.79-1.17 (m, 6H, $CH_2CH_2CH_2$); ^{13}C NMR (75 MHz, CD_3OD): δ 174.7 (COOH), 157.5 (NHC(=O)O), 144.2 (C_{arom}), 141.4 (C_{arom}), 127.6 (C_{arom}), 127.0 (C_{arom}), 125.1 (C_{arom}), 119.8 (C_{arom}), 66.7 (CH_2O), 55.2 (C_{alpha}), 42.7 (Fluorenyl C_9), 38.5 (CH_2NH), 31.5 (CH_3), 29.6 (CH_2), 25.0 (CH_2), 22.9 (CH_2); HRMS (FAB) calcd. for $C_{22}H_{26}N_2NaO_6S$ ([M + Na]$^+$) 469.1409; found: 469.1398.

Peptide synthesis. All peptides were synthesized based on the Fmoc chemistry strategy on a PS3 peptide synthesizer (Protein Technologies Inc., Tucson, AZ, USA). Except N^{α}-Fmoc-N^{ε}-methanesulfonyl-lysine, all other Fmoc-protected amino acids and resins were purchased from Novabiochem®. Four equivalents of Fmoc-protected amino acids were used for the synthesis. All the peptides were cleaved from the resin by reagent K (83.6% (v/v) trifluoroacetic acid, 5.9% (v/v) phenol, 4.2% (v/v) ddH_2O, 4.2% (v/v) thioanisole, 2.1% (v/v) ethanedithiol), precipitated in cold diethyl ether, and purified by reversed-phase high pressure liquid chromatography (RP-HPLC) on a preparative C18 column, eluting with a gradient of ddH_2O containing 0.05% (v/v) of trifluoroacetic acid and acetonitrile containing 0.05% (v/v) of trifluoroacetic acid. The pooled HPLC fractions were stripped of acetonitrile and lyophilized to give all peptides as puffy white solids. Peptide purity (>95%) was verified by analytical RP-HPLC, and their molecular weights were confirmed by either matrix assisted laser desorption

ionization-time of flight (MALDI-TOF) or electrospray ionization (ESI) mass spectrometric analysis.

GST-CBP (1079-1457) expression and immobilization. Following the transformation of the pGEX2T vector containing the open reading frame for CBP 1079-1457 (that harbors the bromodomain only) (a kind gift from Prof. Annick Harel-Bellan) into the *Escherichia coli* strain BL21-CodonPlus (DE3)-RIL, one of the resulting colonies was used to inoculate a 50-mL Luria Broth containing 100 μg/mL of ampicillin, and the culture was grown at 37 ºC for 20 h. This culture was subsequently used to inoculate a 450-mL Luria Broth also containing 100 μg/mL of ampicillin, and the culture was grown at 37 ºC until the optical density (OD) at 600 nm reached 0.8. Fresh 1.0 M isopropyl-1-thio-β-D-galactopyranoside (IPTG) was then added to the culture to a final concentration of 0.15 mM, and the culture was grown at 20 ºC for an additional 20 h. The cells were spun down at 6,000 rpm and 4 ºC for 10 min, and the pellet was resuspended in 10 mL of the cold TBS buffer (50 mM Tris•HCl (pH 8.0), 150 mM NaCl,) that contained 10% (v/v) of triton X-100 and one tablet of protease inhibitor cocktail (Roche Laboratory). Resuspended cells were lysed by double passage through a French pressure cell at 700 psi and centrifuged at 12,000 rpm and 4 ºC for 30 min to remove cell debris. The supernatant was incubated with rotation on a Nutator at 4 ºC for 4 h with 35 mg of the glutathione-agarose that had been pre-swollen in 10 mL of the TBS buffer overnight at 4 ºC and pelleted by centrifugation at 1,400 rpm and 4 ºC for 20 s with the supernatant being discarded. This was followed by centrifugation at 1,400 rpm and 4 ºC for 20 s. The pellet was washed twice, each time with 10 mL of a wash buffer containing 100mM Tris•HCl (pH 8.0), 300 mM NaCl, 10% (v/v) triton X-100, and centrifuged at 1,400 rpm and 4 ºC for 20 s with the supernatant being discarded. The washed pellet was transferred into a 1.5-mL Eppendorf tube and further washed twice, each time with 1 mL of a wash buffer containing 150

mM Tris•HCl (pH 8.0), 450 mM NaCl, and 1/10 tablet of protease inhibitor cocktail, and centrifuged at 1,400 rpm and 4 ºC for 20 s with the supernatant being discarded. The resulting pellet was used to make a 50% (v/v) slurry and kept at 4 ºC for a GST pull-down assay. The concentration of immobilized GST-CBP (1079-1457) was estimated by 10% SDS-PAGE.

GST pull-down assay. Part of the following assay conditions was derived from ref. 1. 195 µL of the above-obtained 50% (v/v) slurry of glutathione-agarose beads with ~80 µg of immobilized GST-CBP (1079-1457) was spun down at 3,000 rpm and 4 ºC for 5 min, and the pellet was washed with 825 µL of a buffer containing 50 mM Tris•HCl (pH 7.5), 0.1% (w/v) BSA, and 1 mM DTT, and centrifuged at 3,000 rpm and 4 ºC for 5 min. To the resulting pellet was added 200 µL of the binding buffer containing 50 mM Tris•HCl (pH 7.5), 50 mM NaCl, 0.1% (w/v) BSA, and 1 mM DTT. 9 µL of a test peptide stock solution (1.2 mM in dd H_2O for all the three test peptides, i.e. the K^{382}-containing, N^{ε}-acetyl-lysine-containing, and N^{ε}-methanesulfonyl-lysine-containing p53 peptide (peptide **1**, **2**, and **3**, respectively on Fig. 3)) was then added and the resulting mixture was rotated on a 360º rotator at room temperature for 2 h before centrifugation at 3,000 rpm and 4 ºC for 5 min. The supernatant was removed and saved for an analytical RP-HPLC analysis of unbound test peptide for the purpose of helping identify the retained test peptide on the immobilized GST-bromodomain (*vide infra*). The pellet was washed with 2x200 µL of the wash buffer containing 50 mM Tris•HCl (pH 7.5), 100 mM NaCl, 0.1% (w/v) BSA, and 1 mM DTT, and centrifuged at 3,000 rpm and 4 ºC for 5 min. The retained test peptide on the immobilized GST-bromodomain was eluted out by thoroughly mixing (vortexing) the pellet with 200 µL of a 1.0 M aqueous HCl solution. After centrifugation at 3,000 rpm and 4 ºC for 5 min, the supernatant was removed and injected into a C18 analytical column for an RP-HPLC analysis of the eluted test peptide. The C18 column was eluted with the

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following gradient of dd H_2O containing 0.05% (v/v) trifluoroacetic acid (mobile phase A) and acetonitrile containing 0.05% (v/v) trifluoroacetic acid (mobile phase B): linear increase from 0% B to 35% B from 0-40 min (1 mL/min), and monitored with UV at 214 nm. This same HPLC condition was also used for analyzing the authentic peptide sample and the unbound peptide from the same incubation experiment (*vide supra*).

Enzymatic Assays. HPLC-based HDAC8 and SIRT1 assays were performed as described previously.[2] For HDAC8 inhibition assay, peptide **2** was used as the substrate and the inhibitor (peptide **3**) had varied concentrations from 0 to 800 μM. The enzymatic reactions were incubated at room temperature for 2 h before quenched with the following stop solution: 1.0 M HCl and 0.16 M acetic acid. HDAC8 was purchased from BIOMOL Research Laboratories, Inc. (Plymouth Meeting, PA). The GST-SIRT1 (whose plasmid is a kind gift from Prof. Tony Kouzarides) was expressed and purified from *Escherichia Coli* as described previously.[3]

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