Supplementary Information for:

$N^\varepsilon$-methanesulfonyl-lysine as a non-hydrolyzable functional surrogate for $N^\varepsilon$-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\(_3\)COOH (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\(_2\)O (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\(_2\)O (5 mL), and the mixture was neutralized at 0 °C while stirring with a 10% (w/v) aqueous Na\(_2\)CO\(_3\) solution. To the obtained solution was added another portion of the 10% (w/v) Na\(_2\)CO\(_3\) 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\(_2\)O (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).
The organics were combined, dried with anhydrous Na₂SO₄, 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): ¹H NMR (300 MHz, CD₃OD): δ 7.69-7.17 (m, 8H, Hₐrom), 4.30-3.90 (m, 4H, Fluorenyl H₉, CH₂O, and Hₐlpha), 2.93 (d, 2H, J=6.9 Hz, CH₂NH), 2.78 (s, 3H, CH₃), 1.79-1.17 (m, 6H, CH₂CH₂CH₂); ¹³C NMR (75 MHz, CD₃OD): δ 174.7 (COOH), 157.5 (NH-C(=O)O), 144.2 (Cₐrom), 141.4 (Cₐrom), 127.6 (Cₐrom), 127.0 (Cₐrom), 125.1 (Cₐrom), 119.8 (Cₐrom), 66.7 (CH₂O), 55.2 (Cₐlpha), 42.7 (Fluorenyl C₉), 38.5 (CH₂NH), 31.5 (CH₃), 29.6 (CH₂), 25.0 (CH₂), 22.9 (CH₂); HRMS (FAB) calcd. for C₂₂H₂₆N₂NaO₆S ([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 Nα-Fmoc-Nε-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) ddH₂O, 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 ddH₂O 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 H2O for all the three test peptides, i.e. the K382-containing, Nε-acetyl-lysine-containing, and Nε-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
following gradient of dd H$_2$O 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.$^3$

**References:**


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