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Enzymatic Activation of a Matrix Metalloproteinase Inhibitor

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Supporting Information

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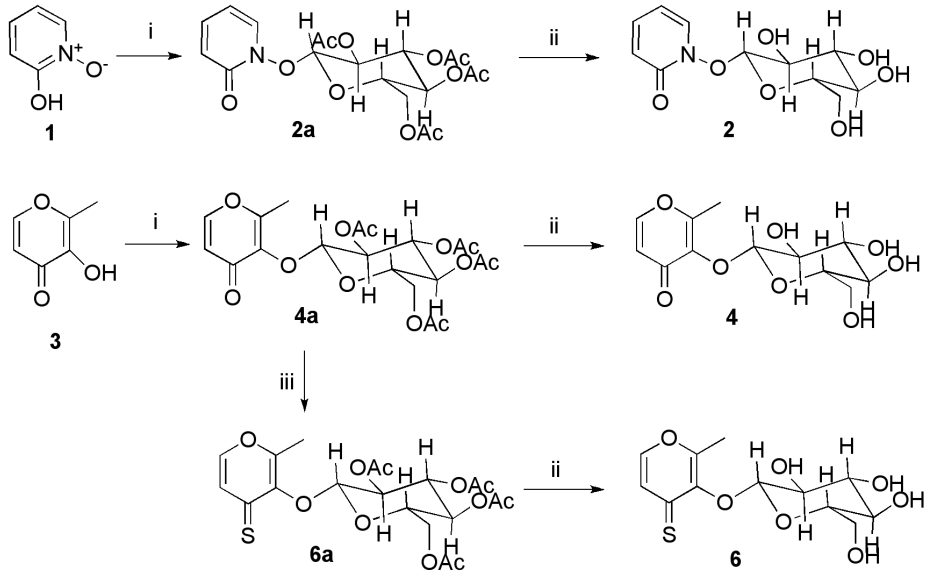
Scheme S1. Synthesis of the glucose-protected ZBGs **2**, **4**, and **6**.[a]



[a] Reagents and conditions: (i) acetobromo-α-D-glucose, tetrabutylammonium bromide, CH_2Cl_2:1.0 M NaOH (1:1), 3 h, room temp; (ii) NaOMe, MeOH, 2-3 h, room temp; (iii) P_4S_{10}, HMDO, benzene, 100 °C, 45 min.

Experimentals

General Methods. All chemicals were purchased from commercial suppliers (Aldrich, Alfa Aesar, TCI, or Fisher) and used as is. β-glucosidase (EC 3.2.1.21) from almonds was purchased from Fluka. ^{1}H and ^{13}C NMR spectra were recorded on either a Varian FT-NMR instrument running at 400 MHz or 500 MHz, or a 500 MHz Jeol instrument at the Department of Chemistry and Biochemistry, University of California, San Diego. Mass spectrometry was performed at the Small Molecule Mass Spectrometry Facility in the Department of Chemistry and Biochemistry at the University of California, San Diego. Elemental analysis was performed by NuMega Resonance Labs, San Diego.

1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyloxy)-pyridin-2(1*H*)-one (2a). 1-Hydroxypyridine-2(1*H*)-one (**1**, 0.50 g, 4.5 mmol) was dissolved in 10 mL of dichloromethane. To this was added 1-bromo-α-D-glucose tetraacetate (0.74 g, 1.8 mmol) and tetrabutylammonium bromide (0.58 g, 1.8 mmol). After heating to 35 °C, 10 mL of 1.0 M NaOH was added. The heterogeneous reaction mixture was vigorously stirred for 3 h. After cooling to room temperature, the reaction was diluted with 20 mL of ethyl acetate then washed 2x with 1.0 M NaOH (20 mL) followed by water and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated for silica gel column purification in 2% MeOH in CH_2Cl_2 yielding an off-white solid in 29% yield (0.23 g). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.51 (dd, J_1 = 6.9 Hz, J_2 = 1.8 Hz, 1H), 7.28 (dt, J_1 = 8.6 Hz, J_2 = 2.9 Hz, 1H), 6.60 (dd, J_1 = 9.8 Hz, J_2 = 1.8 Hz, 1H), 6.04 (dt, J_1 = 6.9 Hz, J_2 = 1.7 Hz, 1H), 5.29 (t, J = 9.2 Hz, 1H), 5.21 (d, J = 8.6 Hz, 1H), 5.16 (t, J = 9.8 Hz, 1H), 5.09 (t, J = 9.8 Hz, 1H), 4.26 (dd, J_1 = 12.6 Hz, J_2 = 5.2 Hz, 1H, CHCH_aH$_b$OAc), 4.09 (dd, J_1 = 12.1 H, J_2 = 2.3 Hz, 1H, CHCH$_a$$H_b$OAc), 3.68 (dq, J_1 = 10.3 Hz, J_2 = 2.3 Hz, 1H, CHCH$_a$H$_b$OAc), 2.16 (s, 3H), 2.02 (s, 3H), 2.00 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ = 170.5, 170.1, 169.9, 169.5, 157.9, 139.3, 137.8, 122.8, 104.3, 103.5, 77.5, 72.3, 69.3, 68.0, 61.4, 20.7. ESI-MS(+): *m/z* 441.77 $[M + H]^+$, 464.06 $[M + Na]^+$.

1-Hydroxypyridin-2(1*H*)-one-β-D-glucopyranoside (2). 2a (0.21 g, 0.48 mmol) was dissolved in 10 mL of dry MeOH. To this was added 0.33 mL (1.42 mmol) of NaOMe (25% in MeOH). The reaction was left stirring for 3 h at room temperature before the addition of ~100 mg cation exchange resin (Biorad

AG-50W-X8, H^+ form) that was allowed to stir for an additional 10 min. The reaction mixture was filtered and the solvents were evaporated for column purification eluting with 10% MeOH in EtOAc. **2** was collected as a white solid in 50% yield (0.063 g, 0.23 mmol). ^{1}H NMR (500 MHz, CD_3OD) δ = 7.97 (dd, J_1 = 6.9 Hz, J_2 = 2.3 Hz, 1H), 7.53 (dt, J_1 = 8.6 Hz, 2.3 Hz, 1H), 6.67 (dd, J_1 = 9.2 Hz, J_2 = 1.7 Hz, 1H), 6.34 (dt, J_1 = 6.9 Hz, J_2 = 1.7 Hz, 1H), 4.99 (d, J = 8.0 Hz, 1H), 3.81 (dd, J_1 = 12.1 Hz, J_2 = 1.7 Hz, 1H, CHCH_aH$_b$OH), 3.68 (dd, J_1 = 12.0 Hz, J_2 = 4.6 Hz, 1H, CHCH$_a$$H_b$OH), 3.44 (t, J = 8.6 Hz, 1H), 3.37-3.30 (m, overlapping peaks, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ = 152.9, 140.6, 138.7, 120.9, 107.4, 106.2, 77.4, 76.2, 72.1, 69.2, 60.8. APCI-MS(+): *m/z* 273.94 $[M + H]^+$. Anal. calcd for $C_{11}H_{15}NO_7$: C, 48.35; H, 5.53; N, 5.13. Found: C, 47.95; H, 5.90; N, 4.86.

2-Methyl-4*H*-pyran-4-one-3-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside) (4a). The synthesis of **4a** was accomplished following the same procedure as that for **2a** using 3-hydroxy-2-methyl-4-pyrone (1.0 g, 7.9 mmol), 1-bromo-α-D-glucose tetraacetate (1.3 g, 3.2 mmol) and tetrabutylammonium bromide (1.02 g, 3.2 mmol) in 20 mL of CH_2Cl_2 and 20 mL of 1 M NaOH at 35 °C. **4a** was collected as an off-white solid in 28% yield (0.401 g, 0.88 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.62 (d, J = 5.7 Hz, 1H), 6.32 (d, J = 5.8 Hz, 1H), 5.32 (d, J = 8.1 Hz, 1H), 5.27 (t, J = 9.2 Hz, 1H), 5.16 (t, J = 8.0 Hz, 1H), 5.09 (t, J = 9.8 Hz, 1H), 4.17 (dd, J_1 = 12.0 Hz, J_2 = 4.6 Hz, 1H, CHCH_aH$_b$OAc), 4.10 (dd, J_1 = 12.6 H, J_2 = 2.3 Hz, 1H, CHCH$_a$$H_b$OAc), dq (3.63, J_1 = 10.4 Hz, J_2 = 1.7 Hz, 1H, C*H*CH$_a$H$_b$OAc), 2.29 (s, 3H, ArCH_3), 2.11 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 173.6, 170.4, 170.1, 170.0, 169.5, 161.2, 153.7, 141.2, 117.2, 99.3, 72.4, 71.7, 71.2, 68.3, 61.5, 20.8, 20.6, 15.2. ESI-MS(+): *m/z* 457.00 $[M + H]^+$, 479.15 $[M + Na]^+$.

2-Methyl-4*H*-pyran-4-one-3-β-D-glucopyranoside (4). The synthesis of **4** was accomplished following the procedure for the synthesis of **2** using 0.15 g (0.33 mmol) of **4a** and 0.23 mL (0.99 mmol) of NaOMe (25% in MeOH) in 10 mL of dry MeOH. **4** was collected as a white solid in 95% yield (0.90 g, 0.31 mmol). ^{1}H NMR (500 MHz,CD_3OD) δ = 7.99 (d, J = 5.2 Hz, 1H), 6.43 (d, J = 5.7 Hz, 1H), 4.79 (d, J = 7.5 Hz, 1H), 3.81 (dd, J_1 = 12.0 Hz, J_2 = 2.3 Hz, 1H, CHCH_aH$_b$OH), 3.65 (dd, J_1 = 12.1 H, J_2 = 5.2 Hz, 1H, CHCH$_a$$H_b$OH), 3.39 (t, J = 9.2 Hz, 1H), 3.36 (t, J = 8.6 Hz, 1H), 3.32 (t, J = 9.2 Hz, 1H),

3.23 (m, 1H, C*H*CH_aH_bOH), 2.45 (s, 3H, ArCH_3). ^{13}C NMR (125 MHz, CD_3OD) δ = 175.9, 163.3, 155.9, 142.3, 116.0, 104.1, 77.2, 76.7, 74.1, 69.7, 61.2, 14.6. ESI-MS(+): *m/z* 288.85 $[M + H]^+$, 311.03 $[M + Na]^+$. HRMS calcd for $C_{12}H_{16}O_8Na$: 311.0737; Found: 311.0742. Anal. calcd for $C_{12}H_{16}O_8Na \cdot H_2O$: C, 43.77; H, 5.51. Found: C, 44.08; H, 5.58.

2-Methyl-4*H*-pyran-4-thione-3-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside) (6a). **4a** (0.20 g, 0.43 mmol) was dissolved in 15 mL of benzene and heated to 80 °C. To this was added P_4S_{10} (0.07 g, 0.16 mmol) and hexamethyldisilyloxane (0.30 mL, 1.43 mmol). The reaction was heated to reflux for 45 min. After cooling to room temperature the reaction was filtered and concentrated for column purification in 1% MeOH in CH_2Cl_2 yielding **6a** as an orange solid in 83% yield (0.17 g, 0.36 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.40 (d, *J* = 5.2 Hz, 1H), 7.15 (d, *J* = 5.2 Hz, 1H), 5.52 (d, *J* = 8.1 Hz, 1H), 5.30 (t, *J* = 9.8 Hz, 1H), 5.24 (t, *J* = 7.5 Hz, 1H), 5.11 (t, *J* = 9.2 Hz, 1H), 4.18 (dd, J_1 = 12.6 Hz, J_2 = 4.6 Hz, 1H, CHC$H_a$$H_b$OAc), 4.12 (dd, J_1 = 12.6 H, J_2 = 2.9 Hz, 1H, CHC$H_a$$H_b$OAc), 3.61 (dq, J_1 = 12.1 Hz, J_2 = 2.3 Hz, 1H, C*H*CH_aH_bOAc), 2.33 (s, 3H, ArCH_3), 2.14 (s, 3H), 2.03 (s, 3H), 2.01 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ = 193.4, 170.4, 170.1, 170.0, 169.5, 158.3, 149.1, 145.7, 128.9, 98.4, 72.3, 71.7, 71.1, 68.4, 61.4, 21.1, 20.6, 15.8. ESI-MS(+): *m/z* 472.63 $[M + H]^+$, 494.94 $[M + Na]^+$.

2-Methyl-4*H*-pyran-4-thione-3-β-D-glucopyranoside (6). The synthesis of **6** was accomplished following the procedure for the synthesis of **2** using 0.16 g (0.34 mmol) of **6a** and 0.23 mL (1.0 mmol) of NaOMe (25% in MeOH) in 10 mL of dry MeOH. **6** was collected as an orange solid in 35% yield (0.035 g, >98% pure by HPLC analysis). ^{1}H NMR (500 MHz, CD_3OD) δ = 7.76 (d, *J* = 5.2 Hz, 1H), 7.23 (d, *J* =5.2 Hz, 1H), 5.00 (d, *J* = 8.1 Hz, 1H), 3.82 (dd, J_1 = 12.1 Hz, J_2 = 2.3 Hz, 1H, CHC$H_a$$H_b$OH), 3.64 (dd, J_1 = 12.1 Hz, J_2 = 5.7 Hz, 1H, CHC$H_a$$H_b$OH), 3.47 (t, *J* = 8.1 Hz, 1H), 3.40 (t, *J* = 9.2 Hz, 1H), 3.31 (t, *J* = 9.7 Hz, 1H), 3.21 (dq, J_1 = 9.8 Hz, J_2 = 2.3 Hz, 1H, C*H*CH_aH_bOH), 3.12 (s, 3H, ArCH_3). ^{13}C NMR (125 MHz, CD_3OD) δ = 193.7, 159.3, 150.2, 147.3, 127.8, 102.8, 77.0, 76.5, 74.4, 69.6, 67.6, 61.2, 15.2. ESI-MS(+): *m/z* 304.78 $[M + H]^+$, 326.96 $[M + Na]^+$. HRMS calcd for $C_{12}H_{16}O_7SNa$: 327.0509, Found: 327.0508. Anal. calcd for $C_{12}H_{16}O_7S \cdot 0.5H_2O$: C, 46.00; H, 5.47; S, 10.23. Found: C, 45.91; H, 5.79; S, 10.78.

***N*-(Biphenyl-4-ylmethyl)-1-hydroxy-6-oxo-1,6-dihydropyridine-2-carboxamide, 1,2-HOPO-2 (7).** This compound was prepared as previously reported (Agrawal, A.; Romero-Perez, D.; Jacobsen, J. A.; Villareal, F. J.; Cohen, S. M. *ChemMedChem*, **2008**, *3*, 812-820). ^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.32 (t, *J* = 6.3 Hz, 1H, N*H*), 7.62 (m, 4H), 7.43 (m, 6H), 7.33 (t, *J* = 7.5 Hz, 1H), 6.57 (dd, J_1 = 9.2 Hz, J_2 = 1.7 Hz, 1H), 6.32 (dd, J_1 = 8.6 Hz, J_2 = 1.8 Hz, 1H), 4.45 (d, *J* = 5.7 Hz, 2H, NCH_2). -APCI-MS(-): *m/z* 319.09 $[M - H]^-$. Anal. calcd for $C_{19}H_{16}N_2O_3$: C, 71.24; H, 5.03; N, 8.74. Found: C, 71.27; H, 5.40; N, 8.84.

***N*-(Biphenyl-4-ylmethyl)-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-1,6-dihydropyridin-6(1*H*)-one-2-carboxamide (8a).** In 10 mL of dry DMF was added **7** (0.10 g, 0.31 mmol), Cs_2CO_3 (0.30 g, 0.94 mmol), and acetobromo-α-D-glucose (0.14 g, 0.34 mmol). The reaction was left stirring at room temperature under N_2 for 24 h. Following evaporation of the solvents, the crude product was brought up in EtOAc and washed once each with water and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated for purification on a silica gel column eluting with 1% MeOH in EtOAc to yield **8a** as a white solid in 80% yield (0.16 g, 0.25 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.75 (d, *J* = 8.5 Hz, 2H), 7.66 (d, *J* = 8.6 Hz, 2H), 7.56 (d, *J* = 8.0 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.35 (t, *J* = 5.8 Hz, 1H), 7.30 (t, *J* = 6.3 Hz, 1H), 6.67 (dd, J_1 = 9.2 Hz, J_2 = 1.7 Hz, 1H), 6.56 (dd, J_1 = 6.9 Hz, J_2 = 1.8 Hz, 1H), 5.52 (d, *J* = 8.6 Hz, 1H), 5.14 (t, *J* = 9.5 Hz, 1H), 4.73 (dd, J_1 =14.4 Hz, J_2 = 6.3 Hz, 1H, NHCH_aH$_b$Ar), 4.68 (t, *J* = 8.0 Hz, 1H), 4.57 (t, *J* = 10.3 Hz, 1H), 4.55 (dd, J_1 = 8.6 Hz, J_2 = 2.9 Hz, 1H, NHCH$_a$$H_b$Ar), 4.31 (dd, J_1 = 12.6 Hz, J_2 = 6.3 Hz, 1H, CHCH_aH$_b$OAc), 3.76 (dd, J_1 = 12.6 Hz, J_2 = 2.3 Hz, 1H, CHCH$_a$$H_b$OAc), 3.56 (dq, J_1 = 8.1 Hz, J_2 = 2.3 Hz, 1H, C*H*CH$_a$H$_b$OAc), 2.09 (s, 3H), 1.98 (s, 3H), 1.94 (s, 3H), 1.62 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ = 170.9, 169.9, 169.8, 169.4, 159.3, 157.7, 143.3, 140.8, 140.7, 138.7, 136.8, 129.1, 128.8, 127.9, 127.4, 127.2, 124.1, 106.9, 101.1, 72.9, 71.6, 69.5, 68.2, 61.4, 43.8, 20.8, 20.6, 20.0. ESI-MS(+): *m/z* 650.92 $[M + H]^+$, 673.22 $[M + Na]^+$.

***N*-(Biphenyl-4-ylmethyl)-6-oxo-1-(β-D-glucopyranosyloxy)-1,6-dihydropyridine-2-carboxamide (8). 8a** (0.086 g, 0.13 mmol) was dissolved in dry MeOH (3 mL) in an ice bath. After stirring at 0 °C for 10 min, 30 μL (0.13 mmol) of NaOMe (25% in MeOH) was added. The reaction was stopped after 1 h

by the addition of a ~100 mg of cation exchange resin (Biorad AG-50W-X8, H^+ form) which was allowed to stir for an additional 10 minutes. The crude product was filtered, concentrated, and purified via silica gel chromatography eluting with 0-1% MeOH in CH_2Cl_2 yielding a white solid in 79% yield (0.05 g, >93% pure by HPLC analysis). ^{1}H NMR (500 MHz, CD_3OD) δ = 7.62 (m, 4H), 7.54 (t, J = 6.9 Hz, 1H), 7.47 (d, J = 8.0 Hz, 2H), 7.40 (t, J = 8.0 Hz, 2H), 7.30 (t, J = 7.5 Hz, 1H), 6.73 (dd, J_1 = 9.2 Hz, J_2 = 1.7 Hz, 1H), 6.51 (dd, J_1 = 6.9 Hz, J_2 = 1.7 Hz, 1H), 5.01 (d, J = 8.0 Hz, 1H), 4.62 (d, J = 14.9 Hz, 1H, NHCH_aH$_b$Ar), 4.45 (d, J = 14.9 Hz, 1H, NHCH$_a$$H_b$Ar), 3.64 (dd, J_1 = 12.0 Hz, 2.9 Hz, 1H, CHCH_aH$_b$OH), 3.37 (m, 2H), 3.28 (t, J = 8.6 Hz, 1H), 3.17 (dq, J_1 = 6.3 Hz, J_2 = 2.3 Hz, 1H, CHCH$_a$H$_b$OH), 3.12 (t, J = 9.8 Hz, 1H). ^{13}C NMR (125 MHz, CD_3OD) δ = 161.9, 158.9, 144.6, 140.7, 140.5, 139.7, 136.8, 128.5, 126.9, 126.7, 122.7, 106.6, 105.8, 77.1, 76.3, 72.3, 69.8, 61.1, 47.2. ESI-MS(+): *m/z* 504.97 $[M + Na]^+$. HRMS calcd for $C_{25}H_{26}N_2O_8Na$: 505.1581; Found: 505.1576. Anal. calcd for $C_{25}H_{26}N_2O_8 \cdot 0.5Na \cdot 2CH_3OH$: C, 58.11; H, 6.14; N, 5.02. Found: C, 57.74; H, 6.31; N, 5.10.

UV-Vis Spectroscopy. Absorption spectra of compounds **1**-**8** were taken on a Perkin-Elmer Lambda 25 UV-visible spectrophotometer. To a 1.0 mL solution at 0.05 – 0.06 mM concentration in HEPES buffer (50 mM, pH 7.5) was added β-glucosidase (16 U for compounds **2**, **4**, and **6**, and 100 U for compound **8**). Spectra were monitored over time either at room temperature for compounds **1**-**7** or at 37 °C for compound **8**.

Calculation of K_m. To a 1.0 mL solution of **8** in HEPES buffer at 25 μM, 50 μM, 75 μM, 100 μM, and 200 μM was added 100 U of β-glucosidase. Spectra were monitored over 4 h at 37 °C. The concentration of product formed, 1,2-HOPO-2 (**7**), was determined using the extinction coefficient for **7** calculated at 346 nm (4279 ± 372 $M^{-1}cm^{-1}$) and was plotted versus time (min). The initial velocity (ν) of each reaction was determined analyzing the linear slope of the first 50 min. The K_m value was calculated from the Lineweaver-Burk plot (1/ν vs. 1/[**8**]) where $-1/K_m$ equals the x-intercept (Figure S10).

Acid Stability. To examine the stability of compound **8** towards acid hydrolysis, the absorption spectra of a 0.05 mM solution of **8** in a 0.1 M aqueous solution of HCl was collected. Spectra were collected at room temperature every hour for a 24 h time period. No change in the spectra was observed (Figure S9).

HPLC. Analytical HPLC was performed on a HP Series 1050 system equipped with a Vydac® C18 reverse phase column (218TP, 250×4.6 mm, 5 μm). Separation was achieved with a flow rate of 1 mL/min and the following solvents: solvent A is 5% MeOH and 0.1% formic acid in H_2O and solvent B is 0.1% formic acid in MeOH. Starting with 95% A and 5% B, an isocratic gradient was run for 15 min to a final solvent mixture of 5% A and 95% B, which was held for 5 min before ramping back down to 95% A and 5% B in 2 min and holding for an additional 4 min. Compounds **1**-**6** were prepared in HEPES buffer (50 mM, pH 7.5) at a concentration of 1.6 mM and compounds **7**-**8** were prepared at a concentration of 1 mM. Retention times of compounds **1**-**8** were determined under identical HPLC conditions prior to evaluation of glucose cleavage of the protected compounds.

To evaluate the efficiency of glucose cleavage for the protected ZBGs **2**, **4**, and **6**, 1 mL samples of each compound were made up at a concentration of 1.6 mM in HEPES buffer (50 mM, pH 7.5). To each sample was added 50 U of β-glucosidase (1 U/μL) and incubated at 37 °C. After 1 h, a 400 μL aliquot was collected and filtered through a microcentrifuge filter (30 kDa MWCO, PLTK cellulosic membrane) to remove β-glucosidase prior to injection on the HPLC. For the full-length inhibitor **8**, a 1 mL sample in HEPES buffer at a concentration of 1 mM was incubated at 37 °C with 250 U of β-glucosidase (1 U/μL) for 5 h. A 200 μL aliquot was collected every hour and filtered through a microcentrifuge filter prior to injection on the HPLC.

Inhibition Assays. MMP-9 (catalytic domain, human, recombinant), MMP-8 (catalytic domain, human, recombinant), and the assay kit were purchased from BIOMOL International. The assays were carried out according to the procedure provided with the kit. MMP activity was measured in 96-well plates using a Bio-Tek Flx 800 fluorescent plate reader. The ZBGs and their protected analogs were dissolved in

DMSO and diluted in HEPES buffer (50 mM, pH 7.5) to a concentration of 10 mM for **1**-**2**, 40 mM for **3**-**4**, and 1.25 mM for **5**-**6**. Each well contained 20 μL of MMP-9 (0.45 U/mL), inhibitor (10 μL fo **1**-**6**), 5 μL of β-glucosidase (1 U/μL) when used, and buffer for a total volume of 99 μL. These were incubated for 1 h at 37 °C. A control sample containing just β-glucosidase (5 μL at 5 U/μL) with MMP-9 (20 μL) was also prepared to confirm that the β-glucosidase did not inhibit of MMP-9. The reaction was initiated by the addition of 1 μL (400 μM) of the fluorescent substrate (Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 where Mca = (7-methoxycoumarin-4-yl)-acetyl and Dpa = *N*-3-(2,4-dinitrophenyl)-L-α-β-diaminopropionyl)) and kinetic activity was measured every minute for 30 min with excitation and emission wavelengths at 335 nm and 405 nm, respectively. Enzyme activity with inhibitor was calculated with respect to the control experiment (no inhibitor present). Measurements were performed in duplicate in two independent experiments.

For **7** and **8**, 1 mL sample in HEPES buffer at a concentration of 1 mM was incubated at 37 °C with 250 U of β-glucosidase (1 U/μL) for 4 h giving a final concentration of 0.8 mM of the inhibitor. After 4 h, a 200 μL aliquot was collected and filtered through a microcentrifuge filter. UV-Vis absorption spectroscopy was to verify that the concentration of 1,2-HOPO-2 was 0.71±0.2 mM in each of the samples using the extinction coefficient for 1,2-HOPO-2 (**7**) calculated at 346 nm (4,279.3 ± 372 $M^{-1}cm^{-1}$). Each well was made up with 20 μL of MMP-9 (0.45 U/mL), inhibitor (2 μL for **7** and **8**), and buffer for a total volume of 99 μL. These were incubated for 30 min at 37 °C. For MMP-8 inhibition assays, each of the samples was diluted to 1 μM in HEPES buffer and 15 μL of each were added to 20 μL of MMP-8 (0.092 U/mL) and buffer for a total of 99 μL. The reaction was initiated by the addition of 1 μL (400 μM) of the fluorescent substrate and kinetic activity was measured every minute for 30 min with excitation and emission wavelengths at 335 nm and 405 nm, respectively. Enzyme activity with inhibitor was calculated with respect to the control experiment (no inhibitor present). A control sample was also performed, in which an aliquot of 250 U of β-glucosidase (1 U/μL) with 1 mL of HEPES buffer was incubated for 4 h at 37 °C, filtered through a microcentrifuge filter, and evaluated in the MMP assay to

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confirm that no inhibition is observed with β-glucosidase. Measurements were performed in duplicate in two independent experiments.

IC_{50} values were obtained for compounds **1-8** against MMP-9 and for compounds **7** and **8** against MMP-8. Serial dilutions of the compounds in DMSO were incubated at 37 °C for 30 minutes with 20 μL of the appropriate MMP (0.45 U/mL for MMP-9 and 0.092 U/mL for MMP-8) and HEPES buffer (50 mM, pH 7.5) for a total volume of 99 μL. The reaction was initiated by the addition of 1 μL (400 μM) of the fluorescent substrate and kinetic activity was measured every minute for 20 min with excitation and emission wavelengths at 335 nm and 405 nm, respectively. Enzyme activity with inhibitor was calculated with respect to the control experiment (no inhibitor present). Measurements were performed in duplicate. The percent inhibition is plotted versus the inhibitor concentration. A linear fit of the data for each experiment gives the IC_{50} value of the inhibitor where y = 50%.

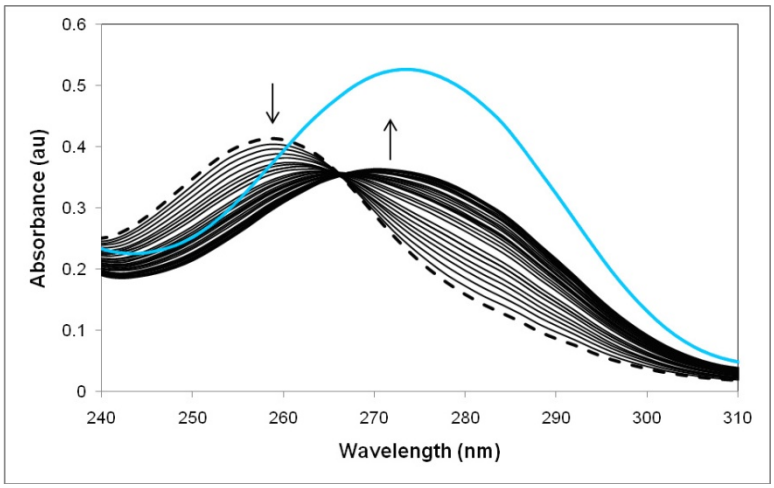

Figure S1. Absorption spectra of the glucose-protected ZBG **4** (0.06 mM in HEPES buffer) in the presence of β-glucosidase (16 U) monitored every minute for one hour with spectra shown every 2 min. The heavy lines are the initial (dashed) and final (solid) spectra; arrows indicate change in spectra over time. An authentic sample of the ZBG (compound **3**, ~0.1 mM) in HEPES buffer is also shown in blue.

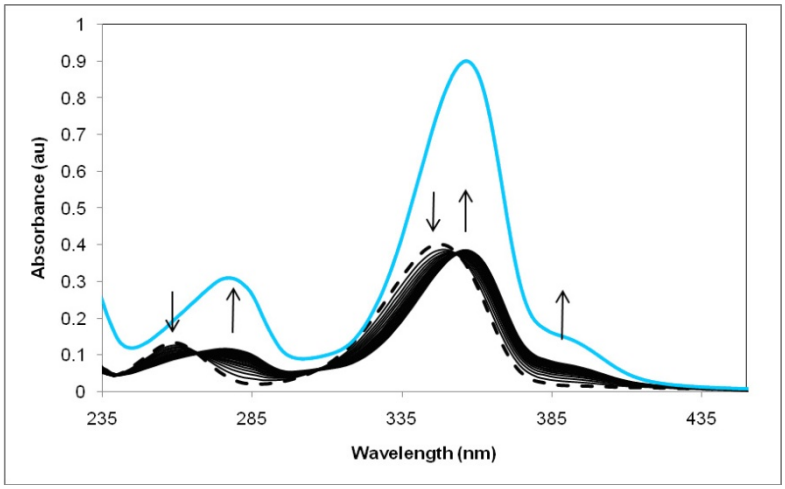

Figure S2. Absorption spectra of the glucose-protected ZBG **6** (0.05 mM in HEPES buffer) in the presence of β-glucosidase (16 U) monitored every minute for one hour with spectra shown every 2 min. The heavy lines are the initial (dashed) and final (solid) spectra; arrows indicate change in spectra over time. An authentic sample of the ZBG (compound **5**, ~0.1 mM) in HEPES buffer is also shown in blue.

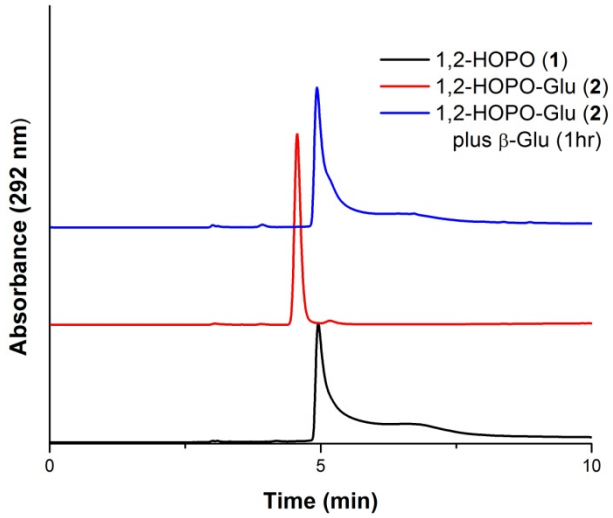

Figure S3. HPLC traces of compounds **1** (black) and **2** (red) and compound **2** after incubation with β-glucosidase (50 U, blue) for 1 h. Retention times are 4.92 min for **1** and 4.42 min for **2**.

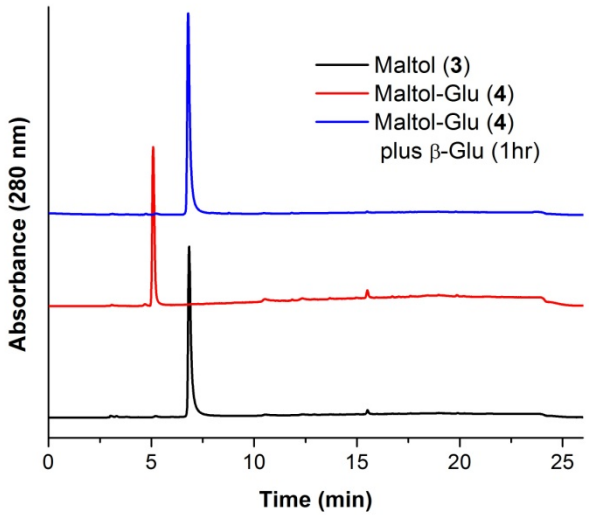

Figure S4. HPLC traces of compounds **3** (black) and **4** (red) and compound **3** after incubation with β-glucosidase (50 U, blue) for 1 h. Retention times are 6.84 min for **3** and 5.07 min for **4**.

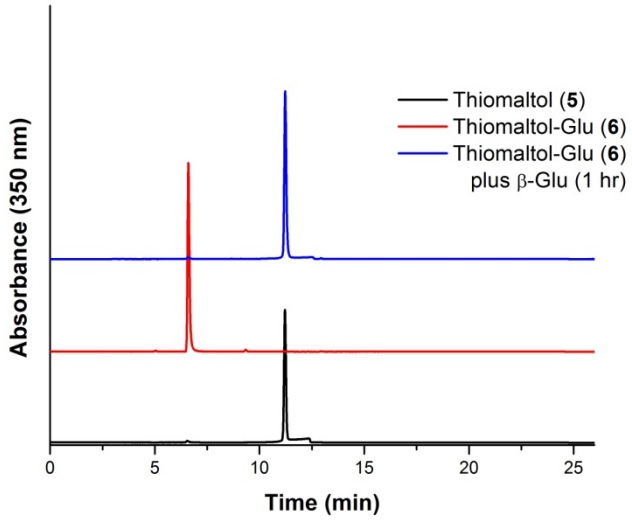

Figure S5. HPLC traces of compounds **5** (black) and **6** (red) and compound **6** after incubation with β-glucosidase (50 U, blue) for 1 h. Retention times are 11.21 min for **5** and 6.60 min for **6**.

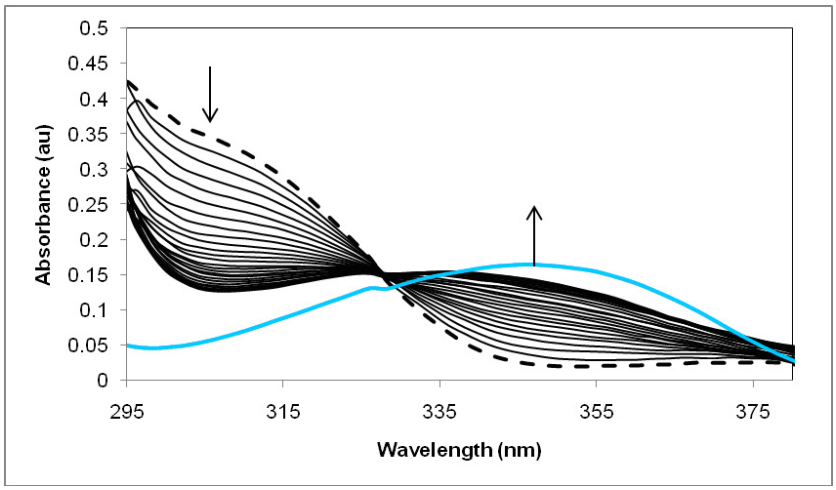

Figure S6. Absorption spectra of the glucose-protected full-length inhibitor 1,2-HOPO-2 (**8**) (0.05 mM in HEPES buffer) in the presence of β-glucosidase (100 U) at 37 °C monitored every 5 min for 4 h with spectra shown every 10 min. The heavy lines are the initial (dashed) and final (solid) spectra; arrows indicate change in spectra over time. A sample of 1,2-HOPO-2 (**7**) (~0.05 mM) in HEPES buffer is also shown in blue.

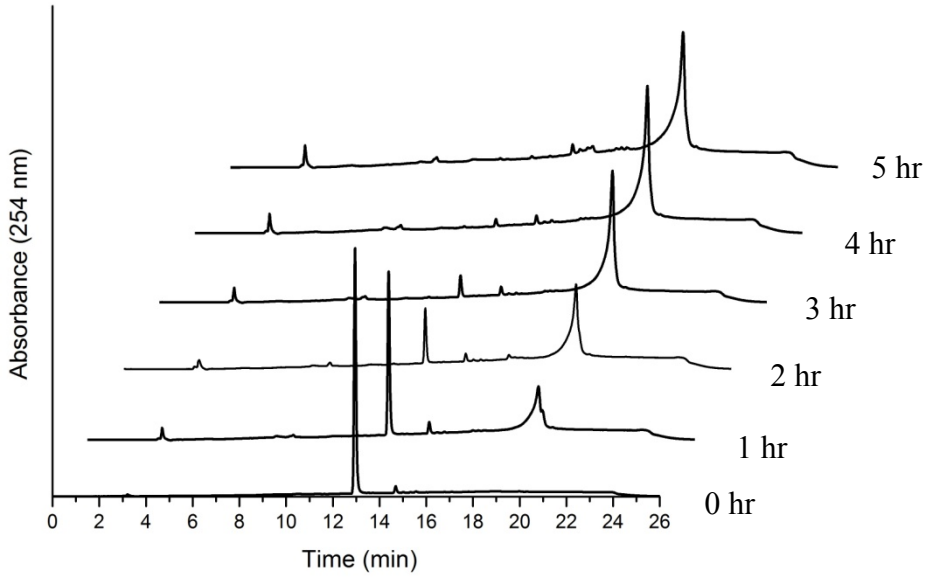

Figure S7. HPLC traces of compound **8** (0.8 mM) over time in the presence of β-glucosidase (250 U). Retention times are 12.88 min for **8** and 19.39 min for the product **7**.

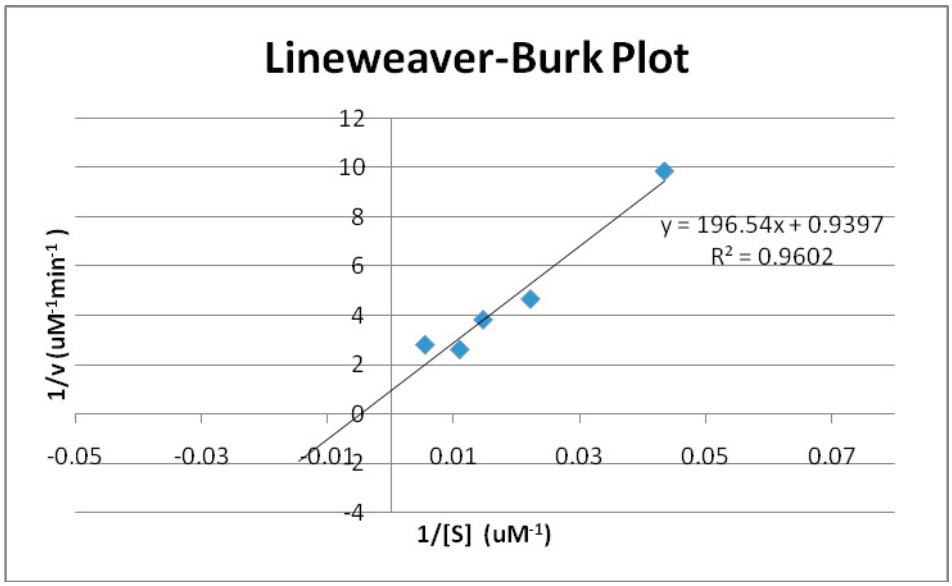

Figure S8. Lineweaver-Burk plot of the initial velocity of β-glucosidase (100 U) cleavage with varying amounts of inhibitor **8**. A K_m of ~210 μM was obtained.

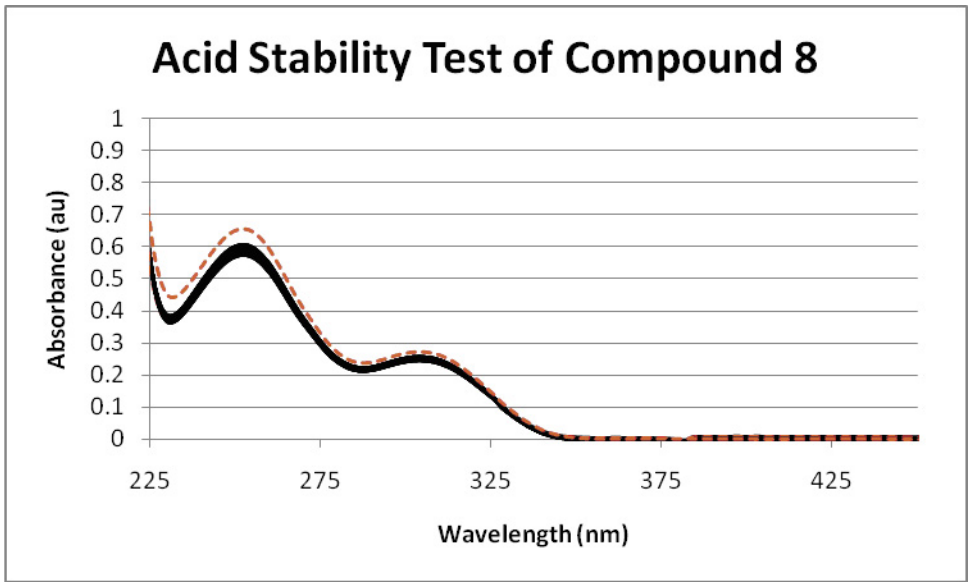

Figure S9. Absorption spectra of the glucose-protected full-length inhibitor **8** (0.05 mM) in 0.1 M HCl monitored every hour for 24 h (overlapping spectra in black). A sample of **8** (~0.05 mM) in HEPES buffer is also shown (red dashed). The overlapping spectra indicate that compound **8** is stable to hydrolysis in the presence of acid.

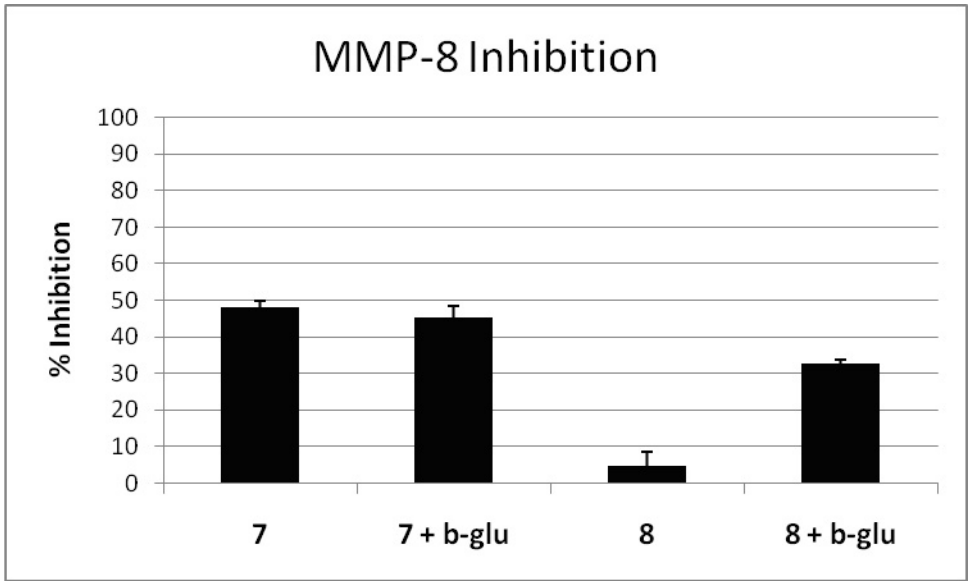

Figure S10. Percent inhibtion of MMP-8 with compounds **7** and **8** tested at 150 nM in the absence and presence of β-glucosidase. Results represent the average of two independent experiments run each in duplicate.