# Supplementary Material (ESI) for Chemical Communications


***Supporting Information***

# Inhibition of Matrix Metalloproteinase-9 by "Multi-Prong" Surface Binding Groups

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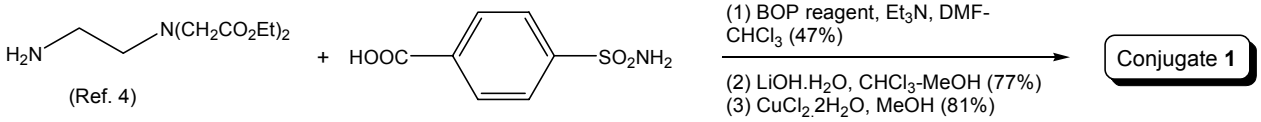
## *Supporting Information*

***Synthesis of the compounds:***

**Conjugate 1**:




To a stirred solution of 4-carboxy benzenesulfonamide (460 mg, 2.28 mmol) in chloroform/DMF (1:1; 20 mL), triethylamine (1.7 mL, 12.2 mmol) was added, followed by the BOP reagent (1.013 mg, 2.29 mmol). After 10 minutes of stirring at room temperature, a solution of the amine.2HCl salt[4] (700 mg, 2.29 mmol) in DMF (5 mL) was added. The reaction was stirred at room temperature for 12 hours and quenched with brine. The organic layer was extracted with chloroform. The combined organic layer was washed with water, dried and evaporated to convert the crude product as viscous oil. This was purified by chromatography ($SiO_2$, 1$^{st}$ only with chloroform, then 3% and 5% MeOH in chloroform) to generate the pure product (450 mg, 47%). $^1$H NMR (400 MHz, $CDCl_3$): δ 1.23-1.27 (t, 6H), 2.93-2.96 (t, 2H), 3.43-3.46 (m, 2H), 3.54 (s, 4H), 4.14-4.19 (q, 4H), 5.20 (br s, 2H), 7.90 (d, 2H, J= 8Hz), 8.01(d, 2H, J=8 Hz), 8.28 (m, 1H).

The above sulfonamide ester (210 mg, 0.51 mmol) in chloroform/methanol (1:1, 10 ml) was treated with $LiOH.H_2O$ (65 mg, 1.54 mmol) for 12 hours at room temperature. Solvent was evaporated, water added, and pH of the solution was lowered to 3 by 2 N HCl. The resulting precipitate was filtered, washed with water and dried to obtain the acid as a white solid (140 mg, 77%). $^1$H NMR (400 MHz, $D_2O$): δ 2.91-2.94 (t, 2H), 3.42-3.45 (m, 2H), 3.48 (s, 4H), 7.87 (d, 2H, J = 8 Hz), 8.00 (d, 2H, J = 8Hz).

To a stirred solution of above sulfonamide acid (66 mg, 0.184 mmol) in methanol (8mL), $CuCl_2.2H_2O$ (31 mg, 0.184 mmol) was added and the reaction mixture was stirred for 6 hours at room temperature. The solvent was removed under reduced pressure. The residue was re-dissolved in ethanol and precipitated with $CH_2Cl_2$. The blue precipitate was filtered, washed with EtOH/$CH_2Cl_2$ (2/10 mL) and dried under vacuum. The final compound was obtained as a blue solid (66 mg, 81%). Anal. Calcd. for $C_{13}H_{15}CuN_3O_7S.H_2O$: C, 35.58; H, 3.90; N, 9.57. Found: C, 35.71; H, 4.06; N, 9.82.

**Conjugate 2**: The synthesis of this compound has been reported recently.[1] Synthetic details are provided in the Supporting Information of this reference.

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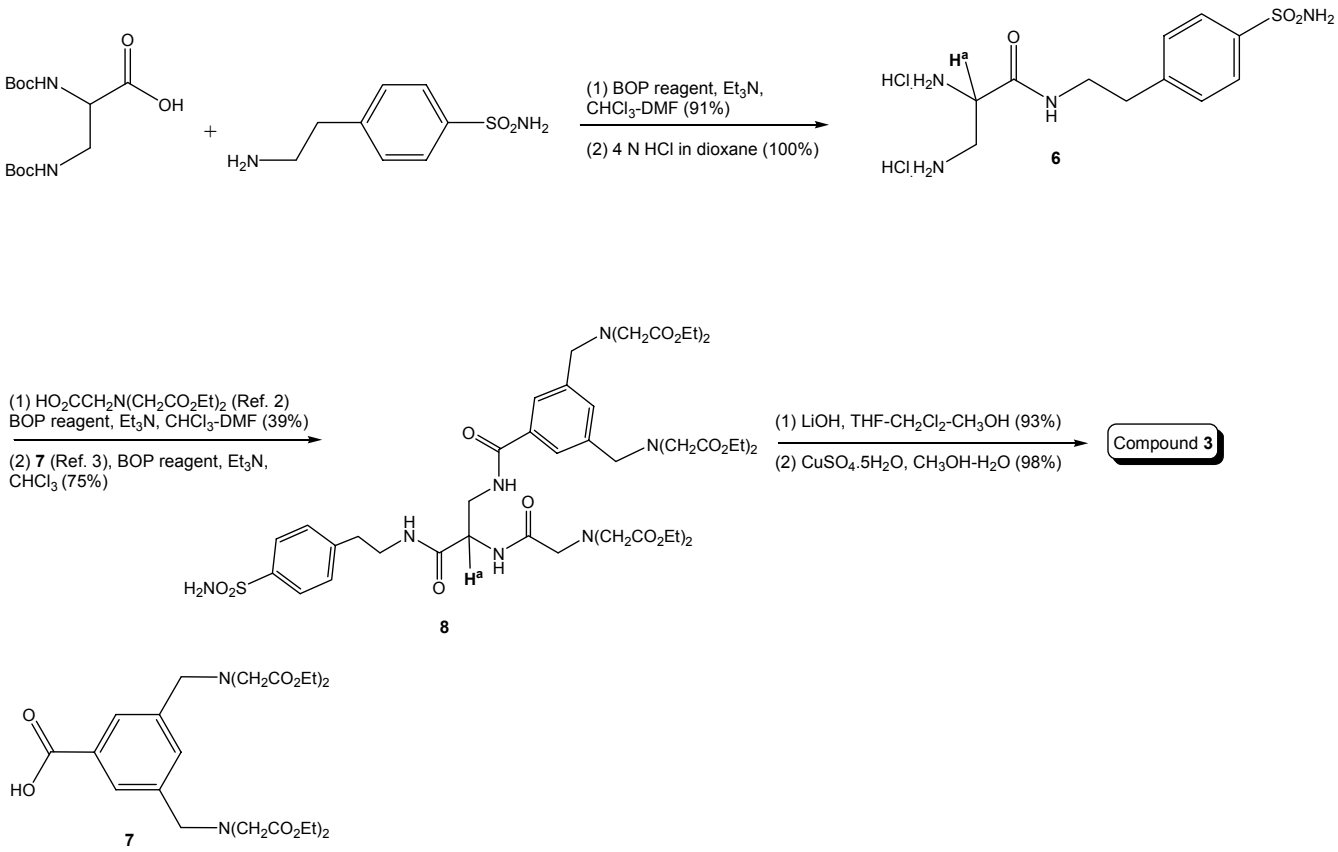
**Conjugate 3:**




**Compound 6**: The diBoc 2,3diaminopropanoic acid (2.50 g, 8.21 mmol), BOP reagent (3.63 g, 8.20 mmol) and $Et_3N$ (2.5 mL, 17.97 mmol) were dissolved in $CHCl_3$ (50 mL) and stirred at room temperature. A solution of 2-aminoethyl benzenesulfonamide (1.65 g, 8.25 mmol) in DMF (20 mL) was added into the above solution. The reaction mixture was stirred at room temperature for 10 h. The reaction was then quenched with saturated brine. The organic solvent was removed under vacuo. The white precipitate was filtered and washed with water. It was used in the next step without further purification. Yield: 3.65 g (91%). $^1$H NMR (300 MHz, $CDCl_3$-$CD_3OD$): δ 2.87-2.91 (m, 2H), 3.11-3.24 (m, 2H), 3.29-3.35 (m, 2H), 4.03-4.15 (m, 1H), 5.69 (bs, 1H), 6.03 (bs, 1H), 7.35 (d, 2H, J = 8.0 Hz), 7.55 (bs, 1H), 7.84 (d, 2H, J = 8.0 Hz).

The above compound (3.0 g, 6.17 mmol) was dissolved in ice-cold 4 N HCl in dioxane (30 mL) and stirred at room temperature for 3 h. The excess solvent was removed under reduced pressure and the residue was then dried under high vacuum. Yield: 2.20 g (100%). $^1$H NMR (300 MHz, $D_2O$): δ 2.93-2.97 (m, 2H), 3.39-3.42 (m, 2H), 3.52-3.61 (m, 2H), 4.22-4.27 (m, 1H), 7.48 (d, 2H, J = 7.9 Hz), 7.83 (d, 2H, J = 7.9 Hz).

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**Compound 8:** To a suspension of **6** (1.50 g, 4.17 mmol) in DMF (40 mL) $Et_3N$ (2.0 mL, 14.37 mmol) was added to dissolve it. Then a solution of diethyl nitrilotriacetate[2] (1.03 g, 4.17 mmol), BOP reagent (1.89 g, 4.27 mmol) and $Et_3N$ (0.7 mL, 5.0 mmol) in $CHCl_3$ (30 mL, 5 mL/h) was added drop-wise to the diamine solution by a syringe pump. After 8 h at room temperature, the reaction was quenched with brine. The product was extracted with $CHCl_3$. The $CHCl_3$ layer was repeatedly washed with water. The crude product was purified by silica gel column chromatography with 8% MeOH in $CHCl_3$ ($R_f$ = 0.1) to provide the pure product as a viscous oil, 1.4 g (39%). $^1$H NMR (300 MHz, $CDCl_3$): δ 1.23-1.29 (m, 6H), 2.63 (bs, 2H), 2.85-2.89 (m, 2H), 3.33-3.40 (m, 2H), 3.47-3.56 (m, 6H), 3.64-3.70 (m, 2H), 4.12-4.17 (m, 4H), 4.51-4.56 (m, 1H), 7.25 (d, 2H, J = 8.5 Hz), 7.45 (bs, 1H), 7.84 (d, 2H, J = 8.5 Hz), 8.12 (bs, 1H).

The coupling between amine (0.30 g, 0.58 mmol) and 2IDA-acid **7**[3] (0.31 g, 0.58 mmol) was carried out in presence of BOP reagent (0.26 g, 0.58 mmol) and $Et_3N$ (0.25 mL, 1.83 mmol) in $CHCl_3$ (35 mL). Stirring was continued for 10 h at room temperature. The work-up procedure was the same as mentioned before for reaction **6** (ester). The pure product was obtained by silica gel column chromatography with 8% MeOH in $CHCl_3$ ($R_f$ = 0.3). Yield: 0.43 g (75%). $^1$H NMR (300 MHz, $CDCl_3$): δ 1.22-1.29 (m, 18H), 2.86-2.91 (m, 2H), 3.30-3.58 (m, 15H), 3.64-3.70 (m, 1H), 3.89 (s, 2H), 3.59 (s, 4H), 4.13-4.20 (m, 12H), 4.52-4.57 (m, 1H), 7.28-7.34 (m, 2H), 7.67 (s, 2H), 7.83 (bs, 1H), 7.88-7.92 (bs, 2H), 8.04 (s, 1H), 8.23 9bs, 1H), 8.56 (bs, 1H).

The structure of the compound **8** was assigned based on the analysis of the $^1$H chemical shift of the hydrogen labeled as $\mathbf{H^a}$. In compound **6**, the chemical shift for this proton is 4.22-4.27 ppm (multiplet). After the coupling reaction with diethyl nitrilotriacetate, the chemical shift of $\mathbf{H^a}$ was found to be shifted to 4.51-4.56 ppm (multiplet). This chemical shift remained unchanged after the reaction with compound **7** in the next step.

**Conjugate 3:** The ester **8** (0.40 g, 04 mmol) was dissolved in $CH_2Cl_2$/THF/MeOH (4/4/8 mL) and solid $LiOH.H_2O$ (0.15 g, 3.5 mmol) was added. The reaction mixture was stirred for 10 h at room temperature. The pH of the solution was lowered to 3 by adding concentrated HCl. The organic solvent was removed under vacuo and the solid was again triturated with absolute EtOH. The residue was washed with THF/ MeOH (1:1). Yield: 0.35 g (93%). $^1$H NMR (400 MHz, $D_2O$): δ 2.91-2.99 (m, 2H), 3.27-3.31 (m, 2H), 3.41 (s, 8H), 3.70 (s, 4H), 3.74-3.79 (m, 4H), 4.02 (s, 4H), 4.42-4.49 (m, 1H), 7. 44-7.52 (m, 2H), 7.87-7.91 (m, 2H), 8.06 (s, 2H), 8.22 (s, 1H). $^{13}$C NMR (100 MHz, $D_2O$): δ 40.19, 44.23, 47.77, 48.12, 49.25, 49.47, 49.81, 50.15, 116.40, 117.23, 121.22, 122.40, 123.99, 127.15, 129.27, 129.45, 162.71, 163.83, 164.92, 165.99.

The above acid (0.20 g, 0.211 mmol) was dissolved in MeOH/$H_2O$ (8/4 mL) in the presence of $NaHCO_3$ (pH = 7.0). Solid $CuSO_4.5H_2O$ (0.159 g, 0.633 mmol) was added to the above solution and stirred for overnight at room temperature. After removal of solvent, the solid was triturated with MeOH, filtered, washed repeatedly with MeOH and then dried. Yield: 0.245 g (98%). Anal. Calcd. for $C_{33}H_{35}Cu_3N_7O_{17}S.3H_2O$: C, 36.75; H, 3.83; N, 9.09. Found: C, 36.89; H, 4.01; N, 8.92.

**Conjugate 4:**

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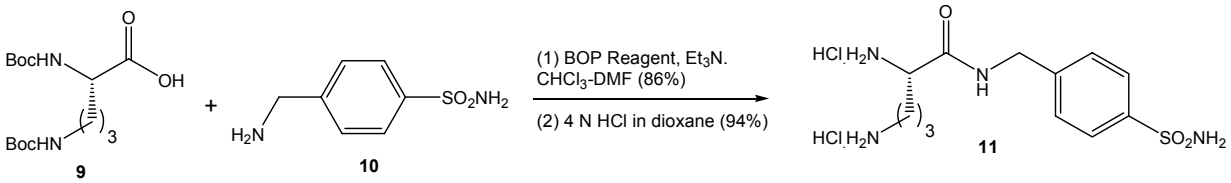


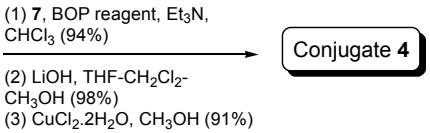


**Compound 11.** DiBoc ornithine **9** (5.0 g, 14.43 mmol) was conjugated with 4-(2-aminomethyl) benzenelsulfonamide **10** (3.2 g, 14.43 mmol) in presence of BOP reagent (6.40 g, 46.0 mmol) and $E_3N$ (6.0 mL, 43.13 mmol) in $CHCl_3$/DMF (60/20 mL). The reaction mixture was stirred for 12 h at room temperature. The work-up procedure was the same as followed before for the reaction **6** (ester). The crude product was purified by silica gel column chromatography with 8% MeOH in $CHCl_3$ ($R_f$ = 0.3) to give a white solid as the product, 6.9 g (86%). $^1$H NMR (400 MHz, $CDCl_3$): δ 1.44-1.62 (m, 21H), 1.78-1.82 (m, 1H), 3.07-3.11 (m, 2H), 4.19-4.23 (m, 1H), 4.48 (s, 2H), 5.13 (bs, 1H), 5.68 (bs, 2H), 7.40 (s, 2H), 7.85 (s, 2H).

The pure product from the above reaction (6.0 g, 10.88 mmol) was dissolved in 25 mL of 4 N HCl in dioxane and stirred at room temperature for 6 h. The work-up procedure was the same as mentioned before for **6**. Yield: 3.83 g (94%).$^1$H NMR (300 MHz, $D_2O$): δ 1.68-1.76 (m, 2H), 1.92-1.98 (m, 2H), 3.00 (t, 2H, J = 7.5 Hz), 4.07 (t, 2H, J = 7.5 Hz), 4.51 (s, 2H), 7.51 (d, 2H, J = 8.0 Hz), 7.88 (d, 2H, J = 8.0 Hz).

**Conjugate 4:** The diamine salt **11** (0.25 g, 0.67 mmol) and 2IDA-acid **7**[3] (0.73 g, 1.33 mmol) were coupled in presence of BOP reagent (0.60 g, 1.35 mmol) and $Et_3N$ (0.5 mL, 3.6 mmol) in $CHCl_3$ (40 mL). The reaction mixture was stirred at room temperature for 12 h. The reaction was quenched with brine. The organic solvent was removed under vacuo. The product was extracted with ethyl acetate. The purification was achieved by silica gel column chromatography with 6% MeOH in $CHCl_3$ ($R_f$ = 0.2) as the eluant. Yield: 0.67 (94%). $^1$H NMR (500 MHz, $CDCl_3$): δ 1.23-1.33 (m, 24H), 1.69-1.79 (m, 2H), 1.86-1.92 (m, 1H), 1.97-2.06 (m, 1H), 3.36-3.42 (m, 1H), 3.47-3.56 (m, 16H), 3.68-3.75 (m, 1H), 3.90 (s, 4H), 3.93 (s, 4H), 4.12-4.20 (m, 16H), 4.42-4.53 (m, 2H), 4.96-4.52 (m, 1H), 5.87 (bs, 1H), 7.10 (bs, 1H), 7.28 (d, 2H, J = 8.2 Hz), 7.48 (s, 1H), 7.52 (s, 1H), 7.67 (s, 2H, J = 8.2 Hz), 7.77 (s, 2H), 7.83 (s, 2H), 7.91 (bs, 1H).$^{13}$C (125 Hz, $CDCl_3$):δ 14.43, 26.16, 30.50, 39.06, 42.98, 52.68, 54.55, 54.64, 57.81, 57.86, 60.81, 60.88, 126.70, 126.93, 127.13, 128.02, 132.63, 132.92, 134.42, 134.89, 139.38, 139.47, 141.66, 143.47, 167.97, 168.38, 171.43, 171.51, 172.57.

To a solution of the above ester (0.455 g, 0.35 mmol) in $CH_2Cl_2$/THF/MeOH (4/4/10 mL) was added solid $LiOH.H_2O$ (0.18 g, 4.28 mmol). The resulting solution was stirred for 12 h at room temperature. The work-up procedure was the same as mentioned before for conjugate **3** (ester hydrolysis reaction). Yield: 0.395 g (98%). $^1$H NMR (500 MHz, $D_2O$): δ 1.75-1.84 (m, 2H), 1.98-1.21 (m, 2H), 3.49-3.52 (m, 2H), 3.96 (s, 8H), 3.99 (s, 8H), 4.46-4.59 (m, 2H), 4.61 (s, 4H), 4.63 (s, 4H), 4.78-4.83 (m, 1H), 7.48 (d, 2H, J = 8.0 Hz), 7.89 (s, 2H), 7.88 (s, 1H), 7.91 (m, 1H), 7.99 (s, 2H), 8.07 (s, 2H). $^{13}$C NMR (125 MHz, $D_2O$): δ 19.52, 27.90, 31.19, 45.26, 57.70, 58.40, 58.58, 58.64, 60.17, 61.04, 128.86, 130.63, 133.41, 134.45, 134.80, 137.86, 138.61, 140.03, 140.33, 142.69, 146.23, 171.46, 171.78, 172.19, 172.22, 176.92.

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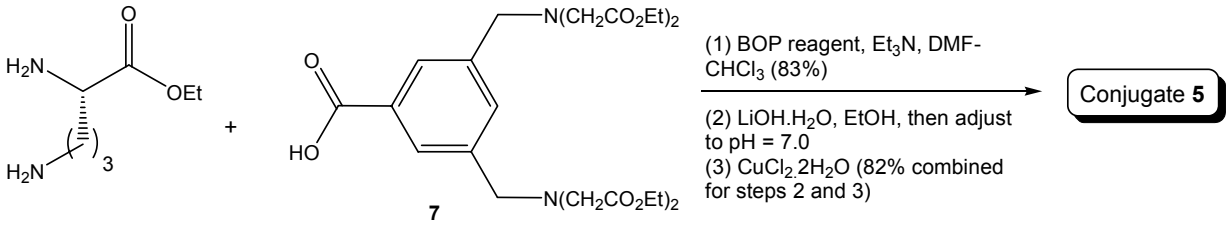
To a solution of the above acid (0.134 g, 0.12 mmol) in MeOH (8 mL) was added $CuCl_2.2H_2O$ (80 mg, 3.40 mmol) and solution was stirred for 6 h at room temperature. The work-up procedure was the same as followed for conjugate **3**. Yield: 0.156 g (91%). Anal. Calcd. for $C_{46}H_{48}Cu_4N_8O_{21}S.4H_2O$: C, 39.26; H, 4.01; N, 7.96. Found: C, 39.42; H, 4.18; N, 8.22.

**Conjugate 5:**




$Et_3N$ (388 µL, 3.845 mmol) was added to a well stirred solution of ornithine ethyl ester (128 mg, 0.549 mmol) in DMF/chloroform (1:1; 20 mL) followed by the addition of BOP reagent (486 mg, 1.098 mmol). After 10 minutes of stirring at room temperature, compound **7**[3] (600 mg, 1.092 mmol) in chloroform (10 mL) was added. The reaction mixture was stirred at room temperature for 12 hours and quenched with brine. The solvents were removed under high vacuum and ethyl acetate was added. The resultant solution was washed with water, 4% $NaHCO_3$ solution and 4% aqueous solution of citric acid. The resulting organic layer was dried ($Na_2SO_4$) and then evaporated. The crude product was purified by column chromatography ($SiO_2$, 1st with 1% and then 2% MeOH in chloroform) affording a colorless viscous liquid (536 mg, 83%). $^1$HNMR (300 MHz, $CDCl_3$): δ 1.23-1.29 (m, 27H), 1.76-1.82 (m,2H), 1.89-1.93 (m, 1H), 2.04-2.08(m, 1H), 3.51-3.54 (m, 18H), 3.93 and 3.95 (overlapping singlet, 8H), 4.12-4.16 (m, 16H), 4.20-4.25 (m, 2H), 4.77-4.81 (m, 1H), 6.81 (t, 1H, J = 8 Hz), 7.09 (d, 1H, J = 8 Hz), 7.50 (s, 1H), 7.55 (s, 1H), 7.79 (s, 2H), 7.82 (s, 2H).

The above ester (315 mg, 0.268 mmol) was treated overnight at room temperature with $LiOH.H_2O$ (199 mg, 4.7 mmol) in absolute ethyl alcohol (10 mL). Solvent was removed and the Li salt of acid was obtained as a white solid. $^1$H NMR (400 MHz, $D_2O$): δ 1.61-1.65 (m, 2H), 1.75-1.78 (m, 1H), 1.86-1.88 (m, 1H), 3.02 and 3.03 (overlapping singlet, 16H), 3.30-3.33 (t, 2H J = 13 Hz ), 3.65 and 3.66 (overlapping singlet, 8H), 4.24-4.27 (dd,1H, J = 5Hz), 7.42 (s, 1H), 7.43 (s, 1H), 7.49 (s, 2H), 7.55 (s, 2H).

The solid acid was redissolved in water and the pH of the solution was adjusted to 7 by $NaHCO_3$ solution. Solid $CuCl_2.2H_2O$ (183 mg, 1.07 mmol) was added to this solution and a color change was observed immediately. Stirring was continued for 6 hours at room temperature. The precipitate was filtered, washed with water, acetone and dried under vacuum to produce conjugate **5** as a blue solid (272 mg, 82% for the last two steps). Anal. Calcd. for $C_{39}H_{40}Cu_4N_6O_{20}.8H_2O.3NaCl$: C, 31.51; H, 3.80; N, 5.65. Found: C, 31.65; H, 3.85; N, 5.71.

***Inhibition Studies:***

The catalytic domains of MMP-9 and MMP-10 utilized herein have been cloned and expressed in *E. coli*, and purified to homogeneity. A detailed account of these protocols will be published elsewhere.

The inhibition studies were performed in 25 mM HEPES buffer, pH 7.5, containing 10 mM $CaCl_2$, 20 µM thiopeptolide Ac-Pro-Leu-Gly-[2-mercapto-4-methylpentanoyl]-Leu-Gly-OEt (TPL), 1 mM DTNB and appropriately diluted enzymes. The reaction was started by the addition of TPL substrate. The time course of

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increase in absorption due to thiolytic cleavage of DTNB was moniotred at 412 nm ($\varepsilon_{412}$ = 13,600 $M^{-1}$ $cm^{-1}$) in the absence and presence of different concentrations of inhibitors. No significant background rate of DTNB reduction or the oxidation of thiols were observed during the course of the steady-state experiments.

Figure S1 shows the influence of imidazole on reversal of inhibition of the MMP-9 catalyzed reaction by conjugate-4. Since imidazole can compete against the surface resident histidine residues of MMP-9 for the binding with IDA-$Cu^{2+}$, it relieves the inhibtiory effect of conjugate-4.

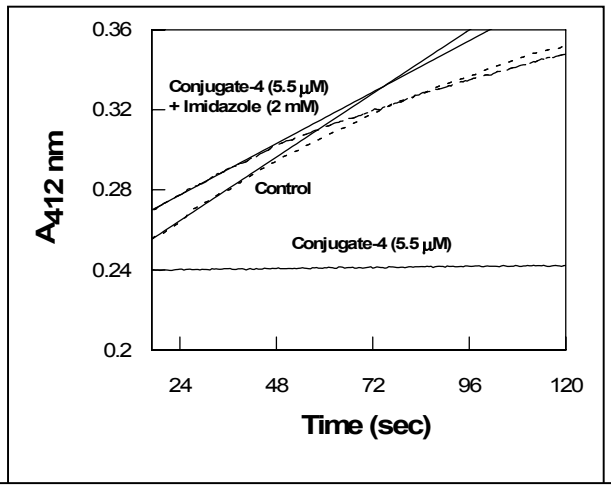


Figure S1: Time courses of the MMP-9 catalyzed reactions in the absence and presence of conjugate-4. The control trace does not include any inhibitor. Note that 5 μM conjugate-4 completely knocks out the enzyme activity. The addition of 2 mM imidazole nearly completely reverses the inhibitory effect of conjugate-4. The solid smooth lines are the linear regression analysis of the reaction traces. The slopes of the control and imidazole containing traces were determined to be 0.0013 and 0.0011 ($\Delta_{A412}$/sec) respectively.

**References:**

(1) B. C Roy, A..Banerjee, M..Swanson, X. G. Jia, M. H Haldar, S. Mallik, D. K. Srivastava, *J. Am. Chem. Soc*. 2004, ***126***, 13206-13207.
(2) E Burks,. N. Koshti, H.. Jacobs, A..Gopalan, *Synlett* 1998, 1285-1287.
(3) B. C. Roy, S. Mallik, *J. Org. Chem.* 1999, ***64*****,** 2969-2974.
(4) Md. A. Fazal,. B. C Roy, S. Sun, S Mallik, K. R. Rodgers, *J. Am. Chem. Soc*. 2001, ***123***, 6283-6290.