Enzymatic activation of a matrix metalloproteinase inhibitor†

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Matrix metalloproteinase inhibitors (MMPi) possessing a glucose protecting group on the zinc-binding group (ZBG) show a dramatic increase in inhibitory activity upon cleavage by β-glucosidase.

Matrix metalloproteinases (MMPs) are a ubiquitous class of zinc(II)-dependent hydrolytic enzymes that have been associated with a wide range of pathologies including cancer, arthritis, heart disease, and stroke.[1–3] Clinical trials of matrix metalloproteinase inhibitors (MMPi) have frequently been hampered by the onset of musculoskeletal syndrome (MSS), which manifests as severe joint pain, and has been attributed to non-specific, systemic inhibition of MMPs and other metalloenzymes.[4,5] One approach to eliminate or minimize MSS and other complications would be to develop prodrugs or 'proinhibitors' that are triggered in a localized fashion, hence restricting inhibitory activity both spatially and temporally. The prodrug concept has been applied to a variety of targets, triggered by stimuli such as light, changes in pH, and enzymatic activity.[6,7]

A common strategy in the development of prodrugs and proinhibitors is through the use of carbohydrate protecting groups. The development of glycoconjugate prodrugs offers the ability to increase water solubility, enhance targeting, and minimize the side effects associated with systemic delivery of cytotoxic drugs.[8–11] The use of glycoside protecting groups, such as glucoside moieties, have been reported for use with targeted treatments such as ADEPT (antibody directed enzyme prodrug therapy) and PMT (prodrug monotherapy).[12–14] The ADEPT approach, developed over 20 years ago, has been widely investigated as a means to deliver drugs specifically to cancerous tissue and has shown promise in both animal studies and clinical trials.[15–17] Glucose-derivatized compounds can also enhance uptake in cancer cells[18–20] and the brain.[9,10,21]

Metalloenzyme inhibitors such as MMPi are particularly suitable to the proinhibitor approach because such compounds generally employ a metal-binding moiety, which if blocked with a protecting group, abolishes inhibitory activity. Specifically, using glucose as a protecting group, a proinhibitor MMPi can be developed to release the active MMPi in the presence of β-glucosidase to restore MMP inhibitory activity. Surprisingly, metalloenzyme proinhibitors have not been widely investigated and very few proinhibitors have been designed for MMPs.[22–29] Only one example of a glycoside MMP proinhibitor has been prepared; however, efforts to release the active MMPi through enzymatic cleavage proved unsuccessful.[27] In this report, we show that an MMPi can be formulated and inactivated as a proinhibitor, deprotected and released by an enzymatic signal, and hence exhibit triggered control over inhibitory activity. The compounds described here allow for spatial and temporal control of MMP inhibition, making them potentially valuable as therapeutics or as chemical tools for studying the roles of MMPs in disease models and developmental biology.[30,31]

Building on the strategy of glycosidic protecting groups, we first focused our attention on the development of glucose-protected non-hydroxamate zinc-binding groups (ZBGs)[32,33] that can be activated by enzymatic cleavage of the protecting group with β-glucosidase to release the ZBG and glucose (Fig. 1). The synthesis of the protected ZBGs (**2**, **4**, and **6**) was accomplished following a literature procedure used by Orvig and coworkers for enzyme-activated metal-binding chelators (Scheme S1).[9,34] The ZBG was protected with acetobromo-α-D-glucose in a 1 : 1 solution of 1.0 M NaOH and CH_2Cl_2 in the presence of $(n\text{Bu})_4\text{NBr}$. The desired products were obtained by cleavage of the glucose acetate groups using NaOMe in MeOH.

To evaluate the ability of these compounds to be enzymatically activated, cleavage of the protected ZBGs in the presence of β-glucosidase (from almond extract, Fluka) was followed using electronic spectroscopy. To a solution of the protected ZBG in HEPES buffer was added β-glucosidase, and the change in absorbance was monitored over time. As can be

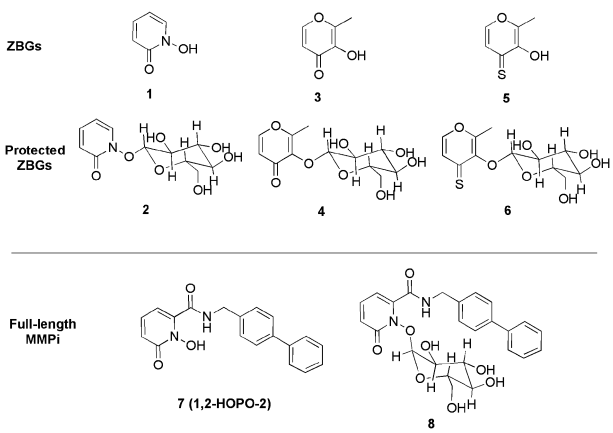

Fig. 1 Structures of inhibitors and proinhibitors tested in this study. Compounds include the ZBGs 1-hydroxy-pyridin-2(1*H*)-one (**1**), 3-hydroxy-2-methyl-4-pyrone (**3**) and 3-hydroxy-2-methyl-4-pyrothione (**5**) as well as the full-length MMPi 1,2-HOPO-2 (**7**).

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† Electronic supplementary information (ESI) available: Synthetic details, characterization of all compounds, and details of cleavage assays. Scheme S1, Fig. S1–S10. See DOI: 10.1039/b923302d

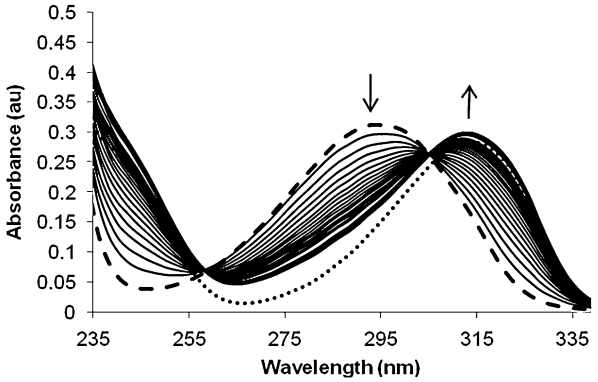
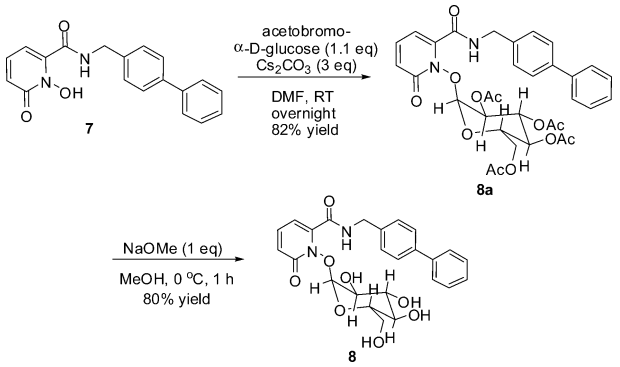


Fig. 2 Absorption spectra of the glucose-protected ZBG **2** (0.05 mM, HEPES buffer, pH = 7.5) in the presence of β-glucosidase (16 U) monitored over time. The heavy lines are the initial (dashed) and final (solid) spectra and arrows indicate the change in spectra over time. The spectrum of an authentic sample of ZBG **1** (dotted) is also shown.

seen in Fig. 2 for compound **2**, the absorbance over time shows a decrease at 292 nm while a band at 312 nm emerges, indicative of the deprotected ZBG 1-hydroxy-2-pyridin-2(1*H*)-one (**1**). Similar spectra were observed for the hydroxypyrone derivatives **4** and **6** (Fig. S1, S2). In addition, cleavage of the protected ZBGs was confirmed by HPLC analysis (Fig. S3–S5). These studies demonstrate that in aqueous buffer at room temperature, the glucose-protected ZBGs can be readily activated in the presence of β-glucosidase providing compelling evidence that hydroxypyridinone and hydroxypyrone ZBGs are well suited for the development of enzyme-activated MMP proinhibitors.

Having demonstrated the use of glucose as an effective protecting group for the aforementioned ZBGs, we aimed to incorporate a glucose protecting group into a full-length MMPi to develop an MMP proinhibitor. We selected the full-length inhibitor 1,2-HOPO-2 (**7**), a potent non-hydroxamate inhibitor of MMPs that uses a 1-hydroxy-2-pyridin-2(1*H*)-one (**1**) ZBG.[35] Synthesis of the MMP proinhibitor was achieved by addition of acetobromo-α-D-glucose and Cs_2CO_3 to **7** in DMF at room temperature to give **8a** in high yields (>80%, Scheme 1). These reaction conditions were a vast improvement in yield over the aqueous reaction conditions used to protect the ZBGs. Surprisingly, these high-yield reaction conditions did not produce the desired products with the ZBGs (**1**, **3**, and **5**). The final proinhibitor (**8**) was obtained by deprotection of the glucose acetate groups with NaOMe in MeOH at 0 °C for one hour.

The MMP proinhibitor **8** was first evaluated for activation by β-glucosidase using electronic spectroscopy and HPLC analysis (Fig. S6, S7). Results from these studies indicate that while cleavage of **8** to produce the active MMPi 1,2-HOPO-2 (**7**) goes to completion, the kinetics of the reaction are noticeably slower than that observed for the protected ZBGs. Complete conversion of **8** to **7** required ~4 h at 37 °C; a K_m value of 210 μM was determined (Fig. S8). Notably, compound **8** was not cleaved under acidic conditions (0.1 M HCl) over 24 h (Fig. S9). Overall, this is the first example of a glucosidase proinhibitor that can be enzymatically cleaved to yield the active MMPi.



Scheme 1 Synthesis of the glucose-protected MMPi **8**.

The ability of the protected compounds to inhibit MMP-9 (gelatinase-B) in the presence of β-glucosidase was evaluated using a fluorescence-based assay.[36] Compounds **1–8** were evaluated at a concentration close to their reported IC_{50} values in the presence and absence of β-glucosidase (Fig. 3). The percent inhibition of MMP-9 with the ZBGs (**1**, **3**, and **5**) is close to 50% when tested with and without β-glucosidase indicating that the presence of low concentrations of β-glucosidase in the assay has little effect on MMP inhibition. The protected ZBGs (**2**, **4**, and **6**) show attenuated inhibition when evaluated without the activating enzyme and complete restoration of inhibition when exposed to β-glucosidase. Compounds **2**, **4**, and **6** do show some inhibition of MMP-9 (Table 1), which is likely due to non-specific binding at the high concentrations of the ZBGs (0.125–4 mM) used in these experiments.

In the absence of β-glucosidase, the complete MMPi **8** (16 μM) displays very little inhibition of MMP-9, but upon activation, MMP-9 activity is inhibited by 73%, which is essentially identical to an authentic sample of inhibitor **7**. Even greater potency was observed against MMP-8, where MMPi **8** could be activated with β-glucosidase to obtain 33% inhibition at only 150 nM (Fig. S10), representing a > 1000-fold increase in activity upon enzymatic activation (Table 1). The factor of 1000 difference in the quotient IC_{50} (IC_{50} value of proinhibitor in the presence and absence of enzyme) has been reported as an optimal value for the ADEPT approach to targeted therapy,[11] and hence MMPi **8** exceeds this threshold with MMP-8. The higher activity against MMP-8 is consistent with the potency of the cleavage product (**7**) against this

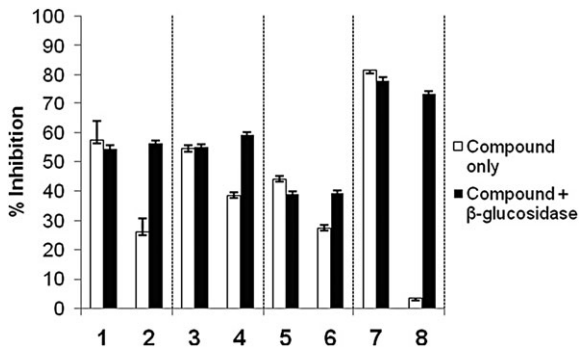


Fig. 3 Percent inhibition of MMP-9 with compounds **1–8** tested at 1 mM for **1** and **2**, 4 mM for **3** and **4**, 125 μM for **5** and **6**, and 16 μM for **7** and **8**, in the absence (white) and presence (black) of β-glucosidase.

Table 1 IC_{50} values of inhibitors and protected inhibitors against MMP-9 and MMP-8 as measured using fluorescence based assay

Inhibitor	IC_{50}	Proinhibitor	IC_{50}	QIC_{50}[a]	Enzyme
1	1.02 mM (± 0.03)	**2**	3.99 mM (± 0.04)	3.9	MMP-9
3	4.03 mM (± 0.05)	**4**	5.7 mM (± 0.3)	1.4	MMP-9
5	138 μM (± 5)	**6**	302 μM (± 5)	2.2	MMP-9
7	2.7 μM (± 0.2)	**8**	587 μM (± 7)	202	MMP-9
7	0.075 μM (± 0.005)	**8**	84 μM (± 1)	1120	MMP-8

[a] QIC_{50} = quotient IC_{50} = IC_{50} proinhibitor/IC_{50} inhibitor.

metalloenzyme.[35] The results for **8** show that incorporation of a ZBG protection strategy into an MMPi gives near complete abolition and recovery of inhibitory activity with these enzyme inhibitors.

In summary, we have demonstrated that it is possible to passivate an MMPi as a proinhibitor, activate it through an enzymatic reaction, and inhibit MMPs in a controlled manner. We have shown that protection of the metal-binding moiety of MMPi can be achieved and enzymatically removed to release an active, intact MMPi. This general strategy of protecting the ZBG with a cleavable group is expected to permit the development of a variety of MMP proinhibitors that respond to a range of stimuli. The ability to selectively utilize MMPi with different chemical and biological triggers will provide a new level of spatial and temporal control over MMP inhibition, which can be useful for both biological studies and therapeutic approaches.

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