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# A High-Affinity Carbohydrate-Containing Inhibitor of Matrix Metalloproteinases

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Matrix metalloproteinases (MMPs) are a class of Zn-containing hydrolases secreted by living cells and which are specialized in endopeptidase activity.[1] MMPs participate in various biological processes such as embryonic development, wound healing, nerve growth, and angiogenesis.[2] Aberrant MMP activities, inducing excessive degradation of the extracellular matrix, are involved in the genesis of diseases such as cancer, rheumatoid arthritis, pulmonary emphysema, and skin ulceration.[3] Since many of these pathologies may benefit from the control of MMP activity,[4] the quest for suitable human MMP inhibitors (MMPIs) has been actively pursued for more than a decade. Many inhibitors endowed with high affinity but modest selectivity, based on a variety of molecular scaffolds, have been reported.[3] Some of these inhibitors have entered into clinical trials for different indications, primarily cancer and arthritis.[5] However, high affinity is often achieved by introducing lipophilic substituents on suitable binding scaffolds, thereby decreasing solubility in water and compromising oral bioavailability.[3] Solubility in water is required for maintaining high drug levels in plasma, which is essential for treatments relying on oral administration. Only very few of the plethora of potentially useful MMPIs reported to date[1,3] are water soluble.[6] Doses of drug higher than those based on intrinsic efficacy must be administered because of limited bioavailability. High doses, in turn, exacerbate the adverse effects of modest selectivity by causing indiscriminate inhibition of other zinc endopeptidases. Furthermore, MMP-related pathologies are usually chronic and any plausible pharmacological scheme would require long-term treatment, during which time lipophilic drugs tend to accumulate in tissues and thereby enhance side effects.[5] High lipophilicity also increases binding affinity to human serum albumin (HSA). Strong binding to HSA has an adverse effect on bioavailability by increasing the half-life in vivo and preventing the drug from reaching the target site.

In the present communication we report a new high-affinity MMP inhibitor that addresses most of the fundamental issues discussed above, using a conceptually novel strategy whereby a glycosidic residue is introduced in the appropriate location of the molecule. The inhibitor is a prototype structure that opens the way to the design of a new class of highly effective MMPIs.

*N*-Isobutyl-*N*-(4-methoxyphenylsulfonyl)glycyl hydroxamic acid (NNGH)[7] is one of the most prominent representatives of a family of inhibitors possessing nanomolar affinity for several MMPs (Scheme 1).[8] The NNGH family of inhibitors suffers from the major drawbacks discussed above, and is therefore inadequate for most applications. The recently published X-ray crystallographic structure of the NNGH–MMP-12 complex[9] allowed us to ascertain that the interaction of the inhibitor with the active site of the enzyme involves binding of the hydroxamate moiety to the catalytic Zn ion and binding of the aromatic group to the $S_1'$ subsite (MMP-12 is the enzyme implicated in the development of emphysema[1]). The isopropyl group on the sulfonamide nitrogen atom points away from the shallow $S_2'$ pocket and does not directly participate in binding. In an effort to overcome the limitations of NNGH, this structural information was used to prepare the new inhibitor **1**, as depicted in Scheme 1, whereby the isopropyl group was replaced with a glucosylated N-hydroxyethyl chain.

The leading concept in designing **1** was to replace a portion of the molecule not directly involved in binding with a water-soluble residue, linked through a spacer of appropriate length, in the hope that its inhibiting properties would not be affected. It must be emphasized that adding a carbohydrate residue to the inhibitor is unprecedented in the NNGH family. The diastereomerically pure β anomer of compound **1** was selectively obtained in good yield (26% over five steps) by reaction of trichloroacetimidate **2** with the hydroxyethyl sulfonamide **3** under Schmidt's glycosylation conditions.[10] Compound **3** was obtained by treatment of the corresponding sulfonamide of the glycine methylester **4** with ethylene oxide and methyl iodide.[11] The synthesis, which produces the glycosidic β isomer exclusively, has been specifically devised as a general method easily amenable to the preparation of the desired derivatives (see below) on a multigram scale through the appropriately substituted imidate.

As expected, compound **1** exhibited a marked increase in water solubility (>30 mM) compared with NNGH, which is essentially insoluble. Even more significantly, the sugar moiety of structure **1** is easily changed to adjust hydrophilicity. Indeed, the solubility of **1** can be scaled down in a stepwise manner by sequentially substituting non-hydrophilic substituents

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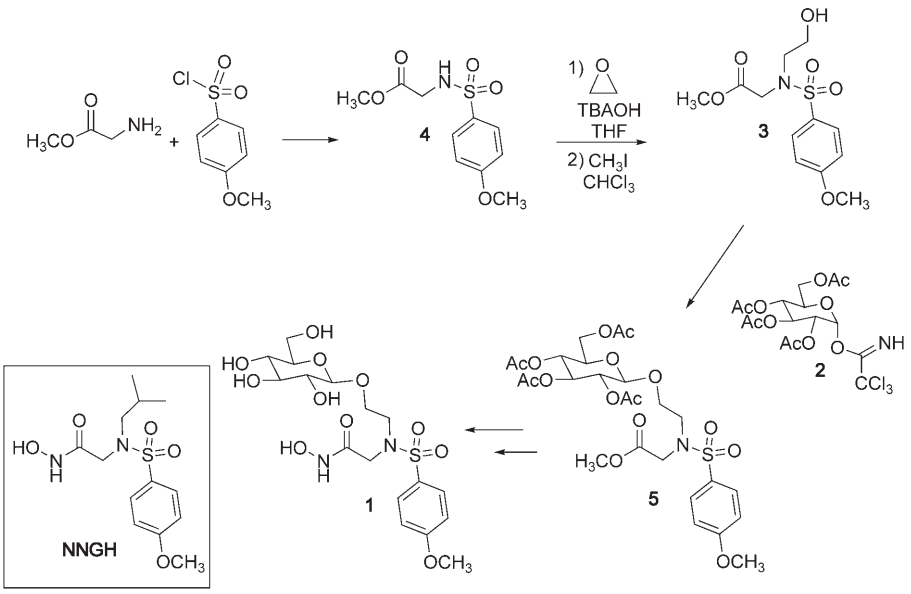
Supporting information for this article is available on the WWW under http://www.chemmedchem.org or from the author: synthetic procedures and characterization details; crystallographic and NMR spectroscopic data; fluorimetric details.




**Scheme 1.** Synthesis of glycosyl inhibitor **1**.

(methyl groups, for example) with hydrogen atoms on the carbohydrate hydroxy groups. The octanol/water partition coefficient (log *P*) is a widely employed descriptor of the hydrophobicity of a molecule and is particularly relevant in drug evaluation. The log *P* value for the parent glucoside **1** and for a number of methyl derivatives have been calculated as Clog *P* values,[12] and compared to the value calculated for NNGH (see Supporting Information). Hydrophilicity decreases more than two orders of magnitude along the set, from −1.76 (hydrophilic) to +0.61 (poorly hydrophobic) depending on the substitution pattern, and the value for NNGH is tenfold smaller than the lowest value (+1.61, substantially hydrophobic).

The inhibition constants of **1** toward several MMPs[13] were evaluated in vitro by a fluorimetric assay.[11] The $K_i$ values obtained are reported in Table 1, and compared with those obtained for NNGH. It can be observed that the affinity of NNGH for several MMPs is preserved for **1**, evidence consistent with a marginal contribution to binding of the glucose moiety. Altogether, the results confirm that the structure modification brought about by glucosidation of the side chain substantially enhanced hydrophilicity without significantly affecting the affinity of the ligand. Remarkably, the affinity is nearly depleted in the absence of the spacer. Indeed, the inhibitor featuring a glucose moiety directly linked to the sulfonamide nitrogen atom had an inhibition constant toward MMP-12 decreased by five orders of magnitude ($K_i$ = 2 mM), presumably because of steric hindrance to binding.

Direct evidence of the lack of significant participation in binding of the glucose moiety was obtained from the X-ray crystal structure of the MMP-12–**1** complex, which was resolved to a 1.8 Å resolution (Figure 1).[11]

The structure is strictly analogous to that of the complex with NNGH,[9] with the hydroxamate moiety chelated to the penta-coordinated Zn and the methoxyphenyl moiety nested inside the $S_1'$ pocket; all the other contacts to the amino acid residues are similarly conserved. Most noteworthy, the glucose residue protrudes out of the protein toward the solvent region located between two symmetry-related protein molecules in the crystal. The lack of strong interactions with the protein residues causes an increased mobility of the glucose moiety, reflected in the lower definition of some of the corresponding atoms, which nonetheless refine to an acceptable B-factor (25 Å$^2$ versus 15–20 Å$^2$ for well-defined atoms).

Despite the marginal interaction with the protein, the presence of the glucose residue appears to be beneficial to selectivity. Indeed, **1** shows an improved selectivity toward MMP-13, which is inhibited over fivefold more effectively than MMP-8, and nearly tenfold more effectively than MMP-12. Selectivity

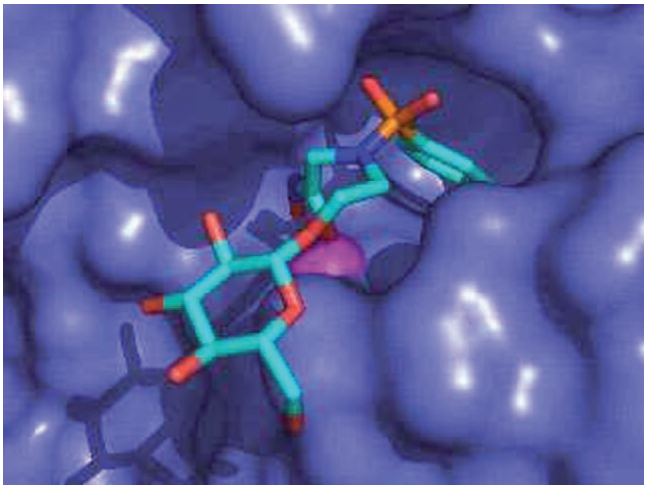


**Figure 1.** X-ray structure of **1** complexed with MMP-12. Only the active site region is shown for clarity.

**Table 1.** Inhibition constants toward several MMPs.[a]

| Inhibitor | MMP-1 | MMP-7 | $K_i$ [nM] MMP-8 | MMP-12 | MMP-13 |
|---|---|---|---|---|---|
| **1** | 286 | 2000 | 9.1 (8) | 14.3 (7) | 1.7 (1) |
| NNGH | 174 | 13000 | 9.0 (7) | 4.3 (1) | 3.1 (1) |

[a] Measured in vitro by fluorimetric assays. Values are averaged over triplicate experiments with errors reported in parentheses.

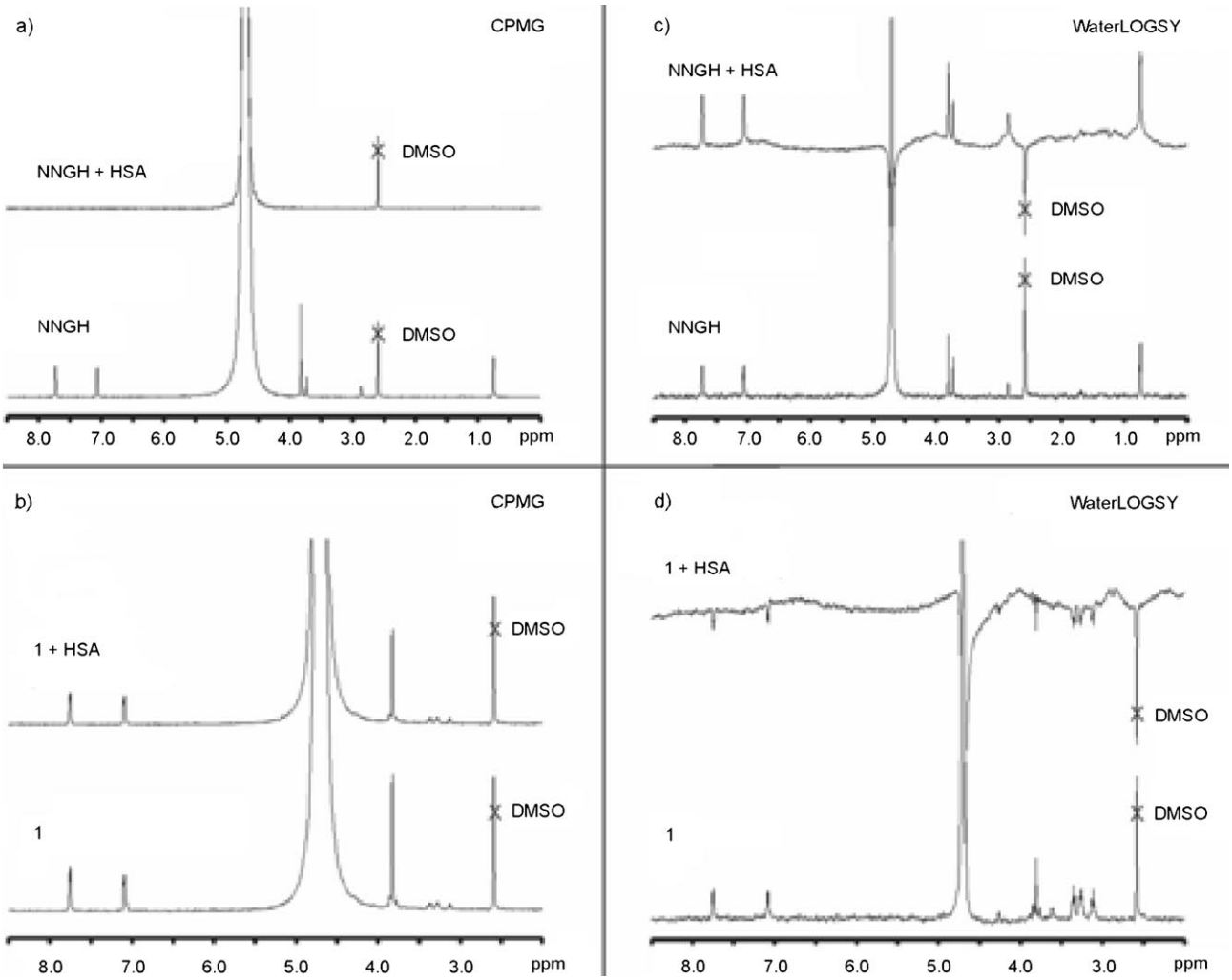



**Figure 2.** a) Relaxation-edited $^{1}$H NMR spectrum of NNGH (200 μM) in the absence and presence of HSA (10 μM). In the presence of HSA, the signals of NNGH decrease in intensity beyond detection. b) Relaxation-edited $^{1}$H NMR spectrum of compound **1** (200 μM) in the absence and presence of HSA (10 μM). The signals of compound **1** are not sizably affected by the presence of HSA. c) One-dimensional WaterLOGSY spectrum of NNGH (200 μM) in the absence and presence of HSA (10 μM). The positive signals of NNGH indicate binding to HSA. d) One-dimensional WaterLOGSY spectrum of compound **1** (200 μM) in the absence and presence of HSA (10 μM). The negative signals of **1** indicate lack of significant binding to HSA.

may therefore constitute an extra bonus from the glycosidation of the inhibitor.

Finally, the interaction with human serum albumin (HSA) was investigated by NMR spectroscopy through CPMG and WaterLOGSY[11] experiments (Figure 2). Both techniques showed unequivocally that **1** interacts very poorly with HSA, whereas NNGH is strongly bound under the same experimental conditions. Gratifyingly, the glucose moiety discourages binding to HSA but not to MMPs.

In summary, glucoside **1** is a new carbohydrate-based inhibitor, unprecedented in the NNGH family, that displays nanomolar affinity toward several MMPs and distinct selectivity for MMP-13. Compound **1** is water soluble and does not appreciably interact with HSA. A distinct advantage of **1** is the modular adaptivity of the design to the desired hydrophilicity, which can be appropriately tuned to specific applications by simply varying the number and the nature of substituents on the carbohydrate residue, through a general synthetic pathway.

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[12] Log*P* (o/w) can be calculated with the CLOG*P* program, available online at the Daylight Chemical Information System site: http://www.daylight.com/daycgi/clogp.
[13] Catalytic domains of MMP-1, MMP-7, MMP-8, MMP-12, and MMP-13 (Cat. N. 101001-13C) were provided by ProtEra s.r.l.

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