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Efficient Synthesis of 5-Amido-3-hydroxy-4-pyrones as Inhibitors of Matrix Metalloproteinases

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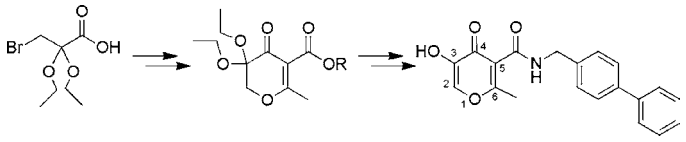
ABSTRACT



3-Hydroxy-4-pyrones are a class of important metal chelators with versatile medicinal applications. An efficient pathway for the preparation of new 5-amido-3-hydroxy-4-pyrone derivatives has been developed. The synthesized 5-amido-3-hydroxy-4-pyrones have been evaluated as inhibitors of matrix metalloproteinases.

3-Hydroxy-4-pyrones (also referred to as hydroxypyrones hereafter) are an important class of biologically active compounds. They can be readily converted to analogues such as hydroxythiopyrones and hydroxypyridinones (3,4-HOPOs) (Figure 1). As charge delocalization is possible within the

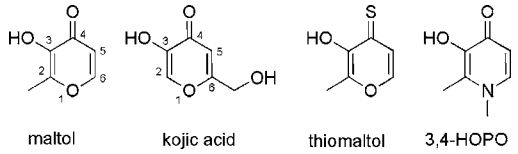


Figure 1. Examples of hydroxypyrone, hydroxythiopyrone, and hydroxypyridinone chelators.

heterocyclic ring, the exocyclic keto group, and the ortho oxyanion derived from the hydroxyl group can efficiently bind to a variety of di- and trivalent metals by forming a five-membered chelate ring.[1] Many reports demonstrate that hydroxypyrones and their thiopyrone and pyridinone analogues are efficient chelators for metal ions such as Fe(III),[1−7] Al(III),[8−11] Ga(III),[3,7,9,12,13] In(III),[3,12,13] Zn(II),[14−17] Cu(II),[15] Ni(II),[15,18] Pb(II),[19] Ru(II),[20] and $[VO]^{2+}$.[17,21−25] The tight

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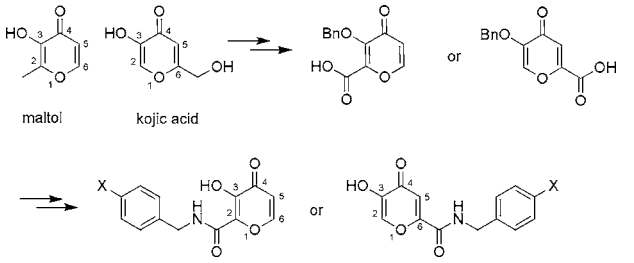
metal binding affinity of these hydroxypyrones and their analogues, as well as the high bioavailability and favorable toxicity profile suggested by maltol (3-hydroxy-2-methyl-4-pyrone)[1] and kojic acid (6-hydroxymethyl-3-hydroxy-4-pyrone),[26] has led to their exploration in medicinal inorganic chemistry. Potential medicinal applications reported in the literature include iron imbalance in anemia and iron overload disorder,[4−6] aluminum removal in Alzheimer's disease,[8−11] treatment of diabetes,[21,23−25] contrast agents for medical imaging,[27] and regulation of metalloenzyme activity.[28−30] Previous hydroxypyrone derivatives related to such investigations were synthesized by structural modification of commercially available maltol and kojic acid (Figure 1),[1,26] which are natural products and can be manufactured by biosynthetic methods from glucose. Because of the versatile coordination chemistry and potential chemotherapeutic application of hydroxypyrones and their thiopyrone and pyridinone congeners, development of new synthetic strategies to access diverse hydroxypyrone derivatives is highly desirable.

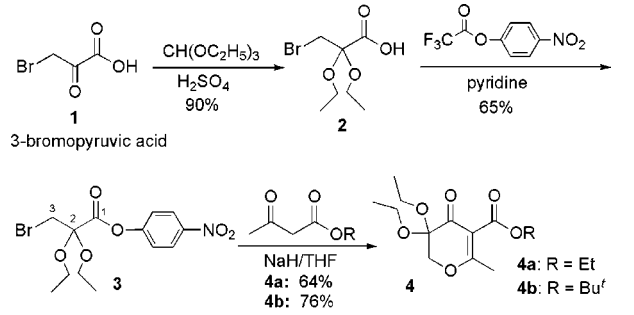
Our laboratory has a particular interest in the development of hydroxypyrone-based matrix metalloproteinase (MMP) inhibitors.[29,30] MMPs are a class of hydrolytic zinc-dependent enzymes that catalyzes peptide bond hydrolysis. They are involved in tissue remodeling, wound healing, and growth.[31] Misregulated activity of these enzymes is also implicated in a variety of diseases such as cancer, arthritis, atherosclerosis, and heart diseases.[32−34] Thus, development of inhibitors for regulation of MMP activity has great therapeutic value.[32−34] We have found that maltol and thiomaltol are more effective chelating inhibitors for MMP-3 (stromelysin) than the widely reported hydroxamate ligands.[29,35] Furthermore, significant improvement of inhibition potency and selectivity of such pyrone-based chelators can be achieved by introduction of a peptidomimetic backbone on the pyrone ring, which leads to enhanced interactions between the MMP subsites and the inhibitor.[30] However, structural modification of maltol and kojic acid only provides access to a group of 2- and 6-amido-substituted hydroxypyrones (Scheme 1). No routes to manipulating maltol or kojic acid in the remaining 5-position have been reported in the literature.[1,5,30,36] As diverse substitution patterns on the pyrone ring may modulate the potency, selectivity, binding mode, and biocompatibility of the inhibitors, our interest in developing pyrone-based MMP inhibitors prompted us to synthesize 5-amidohydroxypyrones to access new potential MMP inhibitors. Analysis of the impact of substituent patterns on inhibitor activity should provide structure−activity information and guidance for further structural optimization of our inhibitor design. Herein, we report the synthesis of 5-amido-3-hydroxy-4-pyrones and their activity as MMP inhibitors.

Scheme 1. Synthetic Path for Pyrone-Based Inhibitors from Maltol and Kojic Acid



The preparation of 5-amido-3-hydroxy-4-pyrones started with commercially available 3-bromopyruvic acid **1** (Scheme 2).[37] Reaction of **1** with triethyl orthoformate in the presence of concentrated H_2SO_4 as a catalyst provided 3-bromo-2,2-diethoxypropionic acid **2** in 85−90% yield. The carboxylic acid intermediate **2** was then transformed to the activated

Scheme 2. Synthesis of 5-Substituted 3,3-Diethoxypyran-4-ones



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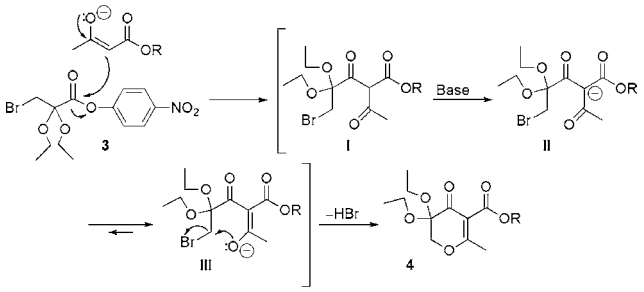
ester 4-nitrophenyl 3-bromo 2,2-diethoxypropionate **3** in 60−65% yield by treatment with 4-nitrophenyl trifluoroacetate in the presence of pyridine. 5-Substituted 3,3-diethoxypyran-4-ones **4a** and **4b** were obtained by refluxing intermediate **3** with a β-keto ester anion in NaH/THF. The yields for this cyclization were in the range of 64−76%. Two 3,3-diethoxypyran-4-ones **4a** (R = Et) and **4b** (R = Bu^t) were prepared as they provide different choices for the subsequent hydrolysis conditions.

The mechanism for the formation of 3,3-diethoxypyran-4-ones **4** is rationalized in Scheme 3. Ketalization of the

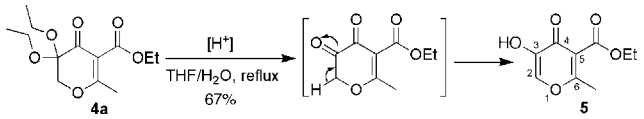
Scheme 3. Formation of 3,3-Diethoxypyran-4-one



2-keto group by two ethoxyl groups results in decreased nucleophilicity and a steric increase on the 3-bromo carbon of intermediate **3**. Thus, the intermolecular nucleophilic reaction between compound **3** and the β-keto ester anion first takes place at the activated ester carbonyl group forming bromo intermediate **I**. In the presence of excess base, the α-carbon on intermediate **I** is further deprotonated leading to the formation of intermediate **II**. An intramolecular nucleophilic reaction at the bromo carbon is favorable under refluxing conditions in THF via a six-membered intermediate **III** which results in the formation of 3,3-diethoxypyran-4-one **4**.[38]

The deprotection of the ketal group in **4** is straightforward by treatment with formic acid or trifluoroacetic acid (TFA) in the presence of moisture (Scheme 4). For example,

Scheme 4. Deprotection of 3,3-Diethoxypyran-4-one



refluxing of **4a** in THF with formic acid provided hydroxypyrone ester **5** in 67% yield. The 5-ester and the 6-methyl groups of compound **5** provide synthetic handles for further functional extension at these positions. When compared with maltol and kojic acid (Scheme 1), **5** should be a versatile synthon for the preparation of pyrone derivatives with unprecedented substitution patterns.

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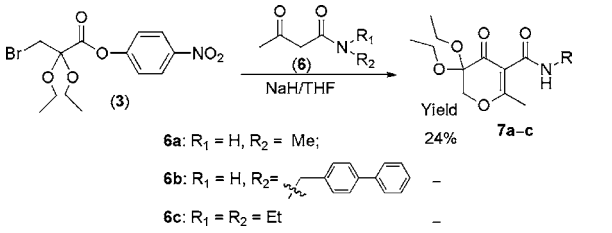
Our interest in the development of 5-amido-3-hydroxy-4-pyrone MMP inhibitors encouraged us to investigate cyclization reactions of activated bromo ester **3** with several β-keto amides **6a**−**c** in an attempt to obtain the corresponding 5-amido 3,3-diethoxypyran-4-ones **7a**−**c** (Scheme 5).

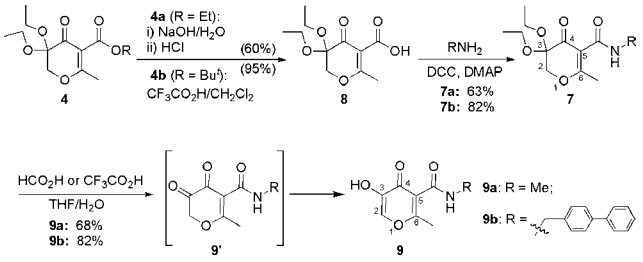
Scheme 5. Cyclization Reactions with β-Keto Amides



Interestingly, the cyclization reaction did not proceed well compared to that of the β-keto ester substrates (Scheme 2). It was observed that the amide substrates **6a**−**c** formed insoluble species under NaH/THF conditions. While reaction of *N*-methyl β-keto amide **6a** provided the expected 5-amidodihydropyrone **7a** in low yield (24%), the reactions of **3** with β-keto amide **6b** and **6c** only resulted in the recovery of starting materials and unidentified compounds without the expected products **7b** and **7c**.

Further investigations generated an improved approach for the synthesis of 5-amido-3-hydroxy-4-pyrones as shown in Scheme 6. Hydrolysis of the ester group of **4** provided

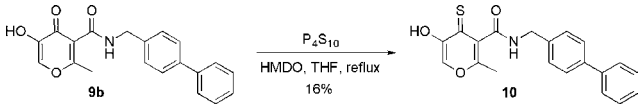
Scheme 6. Synthesis of Compounds **9a,b**



carboxylic acid **8** as a versatile intermediate for the preparation of an amide. The yields for the hydrolysis of ethyl ester **4a** under NaOH/H_2O conditions varied substantially in the range of 30−60%. This outcome may have been due to the sensitivity of the ketal group and the α,β-unsaturated moiety of **4a** under basic reaction conditions and acidic aqueous workup. In contrast, deprotection of *tert*-butyl ester **4b** by TFA was very efficient and selective providing carboxylic acid **8** in 95% yield. Note that the ketal protective group on the pyrone ring can be deprotected by TFA in refluxing THF/H_2O, but the deprotection of the *tert*-butyl ester by TFA in CH_2Cl_2 at room temperature was selective, keeping the ketal group intact. The amide backbone is introduced by coupling the carboxylic acid intermediate **8** with the corresponding amine, forming amide **7** in 63−82% yields. 5-Amido-3-

hydroxy-4-pyrone **9** was obtained in 68−82% yield upon deprotection of the ketal group by refluxing compound **7** in THF in the presence of formic acid or TFA. A thiopyrone analogue **10** was also prepared by thionation of the corresponding pyrone **9b** with P_4S_{10} in refluxing THF in the presence of HMDO (Scheme 7).[39] Several modified conditions were pursued for this reaction, including the use of different solvents (CH_2Cl_2, benzene, dioxane) with or without HMDO, and alternative reagents (Lawesson's reagent); however, none of these adjustments improved the reaction yield. The low yield (16%) of this transformation may be due to a steric effect at the ketone position caused by the neighboring 3-hydroxy and 5-amido groups.[18]

Scheme 7. Synthesis of Inhibitor **10**



The inhibitory activity of biphenyl amides **9b** and **10** against MMP-1 (collagenase), MMP-2 (gelatinase A), MMP-3 (stromelysin), and MMP-9 (gelatinase B) was examined using an established fluorescence-based assay (Table 1).[40]

Table 1. Inhibitory Activity (%) of Compounds **9b** and **10**

compd	MMP-1	MMP-2	MMP-3	MMP-9
9b[a]	21	34	34	33
10[b]	52	94[c]	45	54

[a] Percent inhibition at 100 μM. [b] Percent inhibition at 50 μM. [c] $IC_{50} = 23 \pm 2$ μM.

It was found that both compounds showed relatively poor inhibition against the MMPs evaluated. For hydroxypyrone **9b** at 100 μM concentration, MMP activity was inhibited 21−34%. The hydroxythiopyrone **10** showed more potent inhibition than **9b**. The percent activity of MMP-1, MMP-3, and MMP-9 was inhibited by 52%, 45%, and 54%, respectively, in the presence of 50 μM of **10**. Furthermore, MMP-2 activity was inhibited 94% by compound **10** at a concentration of 50 μM.

Compared with the activity of the previously reported regioisomer **AM-2**,[30] compound **9b** was several orders of magnitude less potent as an MMP inhibitor. This outcome clearly shows that the substitution pattern on the pyrone ring dramatically affects the activity of hydroxypyrone inhibitors. This suggests that the zinc-binding group and not the hydrophobic "backbone" substituent are dictating the orientation of inhibitor binding in the MMP active site. This hypothesis is further supported by the lack of selectivity observed for compound **9b**. **AM-2** strongly inhibits MMP-2 and MMP-3, but not MMP-1; this is likely due to the large biphenyl substituent that can be accommodated in the deep S1′ pockets of MMP-2 and MMP-3, but not in the shallow S1′ pocket of MMP-1.[30] In contrast, **9b** shows no notable selectivity between these three enzymes, indicative of the biphenyl group no longer being directed toward the S1′ pocket. Again, this indicates that the zinc-binding group, and not the biphenyl substituent, is the dominant element dictating the mode of binding. Last, the significant improvement in inhibition potency of compound **10** over **9b** by simply changing the chelator from an O,O to an O,S ligand is consistent with our earlier studies[29] and demonstrates the substantial impact of the zinc-binding group in inhibitor activity.

In summary, the first pathway for the synthesis of 5-amido-3-hydroxy-4-pyrones has been developed. These pyrone derivatives provide new opportunities to explore the metallopharmaceutical applications of these chelators. We are currently exploring the coordination chemistry of these ligands for a variety of bioinorganic applications. These investigations, in combination with the MMP inhibition data from pyrones **9b** and **10**, will provide helpful guidelines for future structural optimization of pyrone-based MMP inhibitors.

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Supporting Information Available: Synthetic procedures, characterization, ^{1}H NMR and ^{13}C NMR spectra, and high-resolution mass spectra (HRMS) of all new compounds; MMP assay experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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