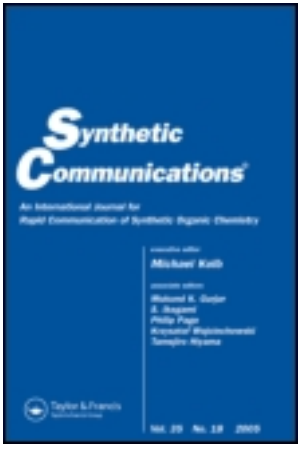

Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lsyc20

An Improved Synthesis of Cyclic Hydroxamic Acids from Gramineae

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Version of record first published: 20 Aug 2006

To cite this article: Kevin Tays & Jeffrey Atkinson (1998): An Improved Synthesis of Cyclic Hydroxamic Acids from Gramineae , Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry, 28:5, 903-912

To link to this article: http://dx.doi.org/10.1080/00032719808006490

SYNTHETIC COMMUNICATIONS, 28(5), 903-912 (1998)

AN IMPROVED SYNTHESIS OF CYCLIC HYDROXAMIC ACIDS FROM *GRAMINEAE*

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Abstract: The boron trichloride sensitive methoxymethyl (MOM) protecting group has been used to efficiently synthesize polymethoxylated derivatives of the benzoxazinone family of cyclic hydroxamic acids. The MOM group allows the unveiling of the hemiacetal at C-2 of these compounds without demethylating ring methoxy substituents, resulting in greatly increased yields.

Cyclic hydroxamic acids found in several of the major cereal crop plants within the family *Gramineae*[1] are now understood to be significant insect toxins that aid in defending the plant against insect attack. They exist in plants as the 2-*O*-β-D-glucosides which are hydrolyzed to the aglucones when the plant tissue is damaged. Studies with the European corn borer[2-4] (*Ostrinia nubilalis* Hübner) have shown that the most abundant cyclic hydroxamic acid in corn, 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-one (DIMBOA), acts as a digestive toxin[5,6] at least in part by inhibiting the proteases within the gut of the insect larvae. DIMBOA has also been noted to be a weak inhibitor of chymotrypsin[7] and a stronger inhibitor of papain.[8] Inhibitory activity towards papain is particularly interesting because in previous work

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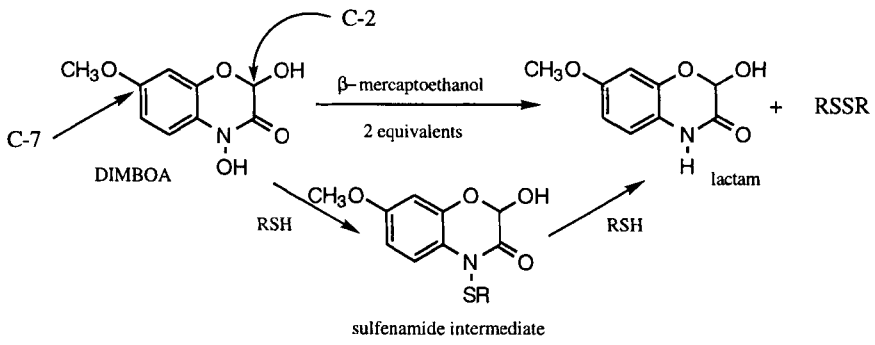


Scheme 1. Reduction of a benzoxazinone cyclic hydroxamic acid such as DIMBOA by excess thiols.

we have determined that the cyclic hydroxamates are susceptible to reduction by low molecular weight thiols.[9,10] The hydroxamic acid group is reduced to the lactam and a disulfide is produced (Scheme 1).

The ease with which particular hydroxamates are reduced is strongly dependent on the substituents on the aromatic ring.[10] In particular, a 7-methoxy group greatly accelerates the rate of reduction, presumably by changing the electronic nature of the hydroxamate nitrogen that is thought to be attacked by thiolate anion. A sulfenamide so produced would react with a further equivalent of thiolate to yield the disulfide and the lactam.

Given the reactivy of DIMBOA with papain we have begun work towards designing novel structures that would act as more powerful inhibitors of this and other thiol proteases. Of particular importance to medicinal chemists are inhibitors of cathepsin B. Cathepsin B has been implicated in a number of disease states including tumor metastasis[11-13], arthritis[14-16] and bone resorption.[17] Kinetic and inhibitory studies

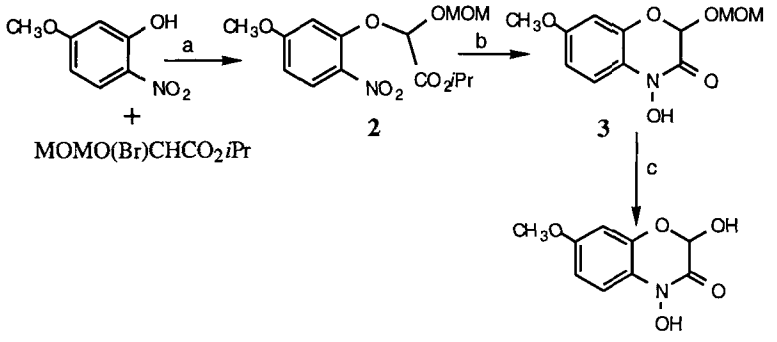
with peptidyl substrates have provided a wealth of information detailing substrate specificity and binding affinity. Cathepsin B has also been recently crystalized[18] and information from this structure has been used to design peptidyl inhibitors such as the acyloxyketones[19-21] and *O*-acylhydroxamates[22,23] of the Syntex group led by Krantz. The *O*-acylhydroxamates have also been shown by ^{15}N-NMR to generate sulfenamide intermediates that covalently link the to the enzyme.[24]

To begin our work we needed an efficient method for synthesizing DIMBOA as well as several polymethoxylated derivatives. The benzoxazinone hydroxamates with multiple oxa-substituents on the aryl ring are expected to react more quickly with an active site thiol given their known reactivities to low molecular weight thiols such as mercaptoethanol.[10] Our preliminary kinetic studies with papain are not in agreement with published kinetic schemes and it is imperative that we know how to control the reactivity of the functional group (the hydroxamic acid) that we hope to include in an inhibitor.

There are several syntheses available for DIMBOA. We have previously used the patented method[25] which requires building the benzoxazinone ring by a reductive cyclization from a derivatized nitrophenol. This is a reasonably efficient method except for the last step which utilizes boron trichloride to dealkylate a methoxy group at C-2 to reveal the hemiacetal. The reagent is not selective to the C-2 methoxy group but will also dealkylate methoxy groups on the aromatic ring. This is particularly problematic for compounds with multiple arylmethoxy groups. It is worth noting that the methyl acetal in question is exceptionally stable to acid hydrolysis by protic acids such as HCl, H_2SO_4, and $HClO_4$. All such attempts resulted in no detectable hydrolysis even under reflux in various solvents, and the slow production of highly

colored materials which were difficult to remove from recovered starting material. In an effort to make the group at C-2 more reactive towards protic or Lewis acids we prepared the 2-*O*-methoxymethylglycolate (2-MOM-glycolate). The rationale was that an extra oxygen atom in the protecting group would make it a more basic site for protonation or complexation. The protected glycolate could be easily prepared by refluxing *iso*-propyl glycolate in dimethoxymethane with catalytic *p*-toluenesulfonic acid and purified by distillation (bp. 78°C, 18 mm Hg). This material was then brominated with NBS using photolytic initiation and the resulting 2-bromo-2-MOM-glycolate coupled to 5-methoxy-2-nitrophenol . The resulting 2-bromo-2-nitrophenoxy-*O*-methoxymethylglycolate, **2**, could be efficiently cyclized to the C-2 protected hydroxamate **3** using a mixture of Pd/C and $NaBH_4$ in aqueous dioxane.[26]

The C-2 MOM group was still stubbornly resistant to protic acid hydrolysis. All attempts to remove the protecting group under such conditions resulted in recovered starting material. Fortunately, this group was considerably more sensitive to the boron trichloride originally used to dealkylate the C-2 methoxy group. The C-2 MOM group of **3** (3.5 g, 13.7 mmol) could be easily removed by treatment of protected materials with 3.2 equivalents of BCl_3 in 150 mL of dry CH_2Cl_2 at -40°C followed by slow warming to -5°C and then quenched with 100 mL of water. Previous methods[25] had used Ag_2CO_3 to assist in the hydrolysis of alkylchloride intermediate, but this was not necessary here. The water was then separated and the organics washed twice with water, brine and then dried with $MgSO_4$. Evaporation gave 2.4 g (83%) of a light beige solid, (m.p. 165-166°C, Lit.[25] 162-163°C). Table 1 lists the yields for both the cyclization and deprotection reactions for various alkoxy-substituted benzoxazine hydroxamic acids and amides. Particular success was achieved in the deprotection of 6,7-dimethoxy and 6,7-methylenedioxy substituted



Scheme 2 (a) dry CH_2Cl_2, reflux, 20 hrs (b) 10:1 $NaBH_4$: Pd/C, 1:1 H_2O:dioxane; HCl (c) 3.2 eq 1.0 M BCl_3 in CH_2Cl_2, -40°C to -5°C over 1 hour.

materials which could only be recovered in 30% yields from the original methodology, but which can now be efficiently prepared.

We are now exploring the kinetics of papain inhibition for those compounds in Table 1 and others and will report on them in due course.

Experimental Section

***i*-Propyl-*O*-Methoxymethylglycolate:** Glycolic acid (50.0 g, 0.66 mmol) was dissolved in 200 ml of *i*-propanol containing 0.5 g of *p*-toluenesulfonic acid. The solution was set to reflux overnight in a Soxhlet extractor containing 4 Å molecular sieves. After cooling, the reaction was poured into 10% Na_2CO_3, the organic layer separated and washed once more with 10% Na_2CO_3, once with brine and dried over anhydrous $MgSO_4$ to give 60 g of a clear light golden liquid. Distillation of this liquid (66 ° C, 20 mm Hg) gave 56.2 g (0.47 mmol, 72% yield) of a clear liquid. Protection of the hydroxyl group of the product*i*-propyl glycolate was accomplished

Table 1. Comparison of the Yields for Deprotection of C-2 Protected Benzoxazinone Hydroxamic Acids

Compound Name	Reaction	% Yields	
		Original[a]	New
2,4-dihydroxy-2H-1,4-benzoxazin-3-one (DIBOA)	Cyclization	83	88
	Deprotection	62	82
2-hydroxy-2H-1,4-benzoxazin-3-one (HBOA)	Cyclization	52	94
	Deprotection	71	90
2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-one (DIMBOA)	Cyclization	60	88
	Deprotection	70	90
2-hydroxy-7-methoxy-2H-1,4-benzoxazin-3-one (HMBOA)	Cyclization	59	79
	Deprotection	90	90
2,4-dihydroxy-6,7-methylenedioxy-2H-1,4-benzoxazin-3-one (DIM_2BOA)	Cyclization	33	84
	Deprotection	38	83
2,4-dihydroxy-7,8-dimethoxy-2H-1,4-benzoxazin-3-one	Cyclization	90	99
	Deprotection	31	74
2,4-dihydroxy-6,7-dimethoxy-2H-1,4-benzoxazin-3-one	Cyclization	30	87
	Deprotection	22	87

a) See references 10 and 25.

by refluxing overnight the crude ester from above with five equivalents of dimethoxy methane in 300 ml of dichloromethane containing 0.5 g of *p*-toluenesulfonic acid. After cooling, the solution was washed twice with 10% Na_2CO_3, dried over $MgSO_4$, and evaporated to give 65.0 g of a clear yellow liquid. Distillation of this material (78°C, 18 mm Hg) gave 60.0 g (77% yield) of a clear colourless liquid.
IR (neat) 2950, 1740 cm^{-1}. MS(EI) m/z (rel inten) 162 (M^+, 1.3), 161 (7.7), 131 (54.1), 103 (48.8), 89 (66.8), 75 (53.2), 61 (62.7), 60 (100). ^{1}H-NMR (acetone-d_6) δ 5.11 (m, 1H), 4.71 (s, 2H), 4.13 (s, 2H), 3.40 (s, 3H), 1.27 (d, 6H). ^{13}C-NMR (acetone-d_6) δ 169.38, 96.15, 68.21, 64.26, 55.42, 21.51

***i*-Propyl-α-bromo-α-methoxymethylglycolate:** This material was prepared fresh before each coupling procedure as it does not store well. A typical procedure prepared 1.5 equivalents of the brominated material based on the nitrophenol to be coupled. In the event, 2.0 g of *i*-propyl-*O*-methoxymethylglycolate (12.3 mmol) was

dissolved in 50 ml of dry CCl_4 under a nitrogen atmosphere. To the stirring solution was added 2.4 g of *N*-bromosuccinimide. A 100 watt incandescent light was placed close to the reaction vessel. After three hours the precipitated succinimide was filtered and the solution of *i*-propyl-α-bromo-*O*-methoxymethylglycolate used immediately.

General procedure for coupling the glycolate to a nitrophenol: A typical coupling procedure involved addition of freshly prepared *i*-propyl-α-bromo-α-methoxymethylglycolate to a stirring solution of the appropriate nitrophenol in dry dichloromethane and refluxing the solution under nitrogen for several hours. When the couplings were complete as judged by TLC (4:1, hexane:EtOAc) the solution was cooled, washed three times with 10% K_2CO_3 to recover the valuable nitrophenol, and evaporated to yield the *i*-propyl-*O*-methoxymethyl-α-(*o*-nitrophenoxy) glycolates as yellow oils which crystallized on standing. These products could be purified by silica gel column chromatography using the same hexane:EtOAc (4:1) solvent system. These materials slowly hydrolyzed on standing to liberate the free nitrophenol and were generally reductively cyclized within a day or two.

General procedure for reductive cyclization: Preparation of the cyclic hydroxamates was performed using a reductive cyclization method first used by Coutts[26] for similar α-(*o*-nitrophenoxy) esters. A typical cyclization involved the dropwise addition of the nitroester (6-9 mmol in 20-25 ml dioxane) to a stirring mixture of 1g $NaBH_4$ and 100 mg of 10% Pd/C in a 1:1 H_2O/dioxane (80 ml) solvent system. Thirty minutes after the addition was complete (~ 2 hours) the catalyst was filtered, the solution was acidified with HCl to pH 3-5 and extracted three times with EtOAc, dried with $MgSO_4$ and evaporated to dryness.

General procedure for deprotection: Deprotection of the C-2 MOM-protected hydroxamtes was accomplished using a 1.0 M BCl_3 solution in CH_2Cl_2. For the synthesis of DIMBOA, 2-*O*-methoxymethyl-4-hydroxy-7-methoxy-1,5-benzoxazin-3-one (MOM-DIMBOA, 3.5 g, 13.7 mmol) suspended in 150 ml of dry CH_2Cl_2 was

cooled to -40 °C using a liquid nitrogen/isopropanol bath under a nitrogen atmosphere. To the stirring mixture was added 3.2 equivalents (48 ml) of 1.0 M BCl_3 solution. The mixture was allowed to warm to -5 °C over one hour and then 100 ml of water was added. After transfer to a separatory funnel the organics were washed twice more with water, dried and evaporated to yield 2.40 g (83 % yield) of a light beige solid. M.p. 165-166 °C, decomposition, (Lit.[25] 162-163 °C, decomposition)

Purity of prepared compounds: All compounds prepared in this work were analytically indistinguishable from those prepared via earlier methods[10] including melting points, IR, ^{1}H- and ^{13}C-NMR, and chromatographed as one spot on silica TLC (10:1, CH_2Cl_2:MeOH)

2,4-dihydroxy-2H-1,4-benzoxazin-3-one (DIBOA): M.p 155-156 °C (decomp), Lit.[10] 155-157°C and[27] 155-156°C. **2-hydroxy-2H-1,4-benzoxazin-3-one (HBOA)**: M.p. 208-208.5 °C, Lit.[28] 201-203°C. **2-hydroxy-7-methoxy-2H-1,4-benzoxazin-3-one (HMBOA)**: M.p. 198-199.5 °C, Lit.[28] 196-198 °C. **2,4-dihydroxy-6,7-methylenedioxy-2H-1,4-benzoxazin-3-one (DIM_2BOA)**: M.p. 151.5-153 °C, Lit.[10] 152-153 °C. **2,4-dihydroxy-7,8-dimethoxy-2H-1,4-benzoxazin-3-one**: M.p. 151-152 °C, Lit. [10] 145-146 °C (decomp). **2,4-dihydroxy-6,7-dimethoxy-2H-1,4-benzoxazin-3-one**: M.p. 152.5-153 °C, Lit.[10] 154-155 °C.

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(Received in the USA 01 September 1997)