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A novel synthesis of 3-(substituted)pyrimido[4,5-*c*]pyridazine-5,7(1*H*,6*H*)-diones

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ABSTRACT

An improved synthesis of 3-(substituted)pyrimido[4,5-*c*]pyridazine-5,7(1*H*,6*H*)-diones, a known subclass of 4-deazatoxoflavins, is reported. The approach involves treatment of 3-methyl-6-(1-methylhydrazinyl)uracil with representative phenyl and alkyl glyoxal monohydrates, which in turn are obtained by selenium dioxide oxidation of the corresponding phenyl and alkyl methyl ketones. The first entry into 4-monosubstituted isomers is also reported.

1,6-Dimethylpyrimido[4,5-*c*]pyridazine-5,7(1*H*,6*H*)-dione, also known as 4-deazatoxoflavin, and its 3,4-disubstituted analogues have been shown to serve as biomimetics of flavin and 5-deazaflavin in their ability to oxidize amines to carbonyl compounds[1] and to abstract hydrogen equivalents from hydrogen donors under certain conditions (Fig. 1).[2] 4-Deazatoxoflavin itself has also demonstrated both inhibitory activity against *Pseudomonas* and DNA-binding properties[3] while not displaying the generalized cytotoxicity that is characteristic of toxoflavin.[4] There are scattered reports of 3,4-disubstituted[3,5] and 3-mono-substituted[2,6] analogues of 4-deazatoxoflavin, but no reports of 4-monosubstituted analogues. The described syntheses of 3-monosubstitued analogues generally proceed in poor yields and via pathways that produce alternate products. Our goal was to develop an improved synthesis that would lead to clean products and possess generality for a wide range of analogues for biological testing.

Our strategy is based on the known reaction of 6-(1-methylhydrazinyl)uracil with glyoxal and α-diketones to yield 3,4-unsubstituted- and 3,4-disubstituted- pyrimido[4,5-*c*]pyridazine-5,7(1*H*,6*H*)-diones, respectively.[3] Despite these prior examples, there are no reports of reaction between this or related 6-(hydrazinyl)uracils and unsymmetrical alkyl or aryl glyoxals. We imagined that reaction could occur by several manifolds. One could proceed via initial hydrazone formation onto either one or both of the aldehyde or ketone moieties, or as is often the case for 6-aminouracils,[7] by nucleophilic attack by the 5-position of the uracil onto either glyoxal carbonyl. Thus, we set out to evaluate a number of reaction conditions to determine if regiochemical preferences for the uracil and glyoxal reaction partners could be established. Initially, we treated 3-methyl-6-(1-methylhydrazinyl)uracil[8] with phenylglyoxal monohydrate in refluxing ethanol or water and obtained the result shown in Scheme 1. Upon cooling a precipitate corresponding to ~85% of **3** was confirmed by ^{1}H NMR and mass spectrometry analysis, and a negative Tollens test for aldehyde. In addition to supporting spectral data, selective hydrazone formation onto the aldehyde moiety under these conditions is well precedented from prior studies on glyoxal hydrazones of methylhydrazine.[9,10] The ~15% co-crystallized impurity plus 5–10% additional material in the mother liquor corresponded to one of two possible cyclization products **4** or **5** by spectral analysis with a distinctive ^{1}H NMR methine proton

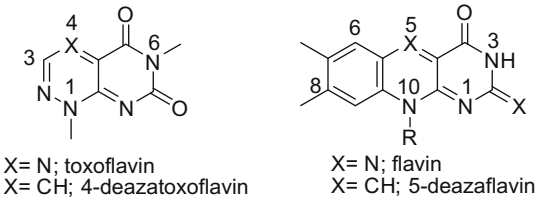

Figure 1. Structure of toxoflavin, flavin and corresponding deaza analogues.

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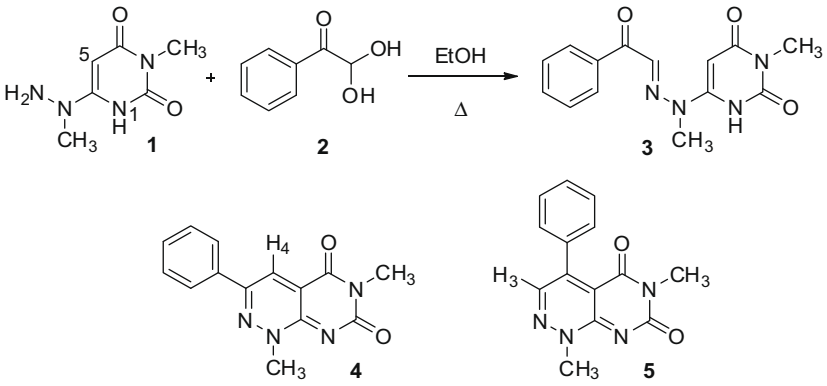


Scheme 1. Reaction products from condensation of (hydrazinyl)uracil **1** with phenylglyoxal **2**.

Table 1
Reaction conditions and product distribution from the treatment of (hydrazinyl)uracil (**1**) with phenylglyoxal monohydrate (**2**)

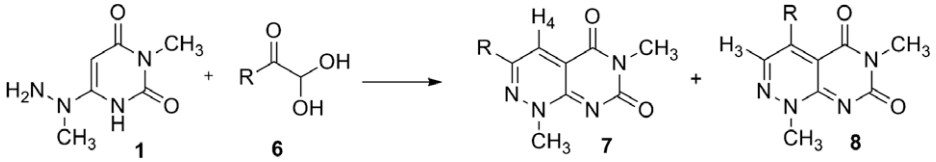
Entry	Reaction conditions	Product (yield)
a	H_2O or EtOH, Δ, 24h	**3** (~50%) & **4** (~25%)
b	H_2O, 2 equiv NaOAc, Δ, 1 h	**4** & **5**; ~1:1 (76%)
c	H_2O, Δ, 2 h (2 equiv NaOAc added after 30 min)	**3** & trace **4**
d	1,2-dichloroethane, Δ, 3 h	**4** (52%)
e	1,2-dichloroethane, $BF_3{\cdot}Et_2O$ Δ, 24 h	**4** (55%)
f	*p*-TsOH, H_2O, Δ, 24 h	**4** (34%)
g	$CH_3OCH_2CH_2OH$, Δ, 24 h	**3** & **4**

(H_4) at δ 8.72 ppm in DMSO-d_6. Ultimately, we generated compound **4** exclusively (vide infra) and determined its structure by single crystal X-ray analysis. This showed the cyclized product to be the 3-phenyl isomer **4**, formed by uracil nucleophilic attack at the C-5 position onto the aldehyde moiety of phenylglyoxal. In an attempt to generate the 4-phenyl isomer **5** directly from hydrazone **3**, various conditions were examined to effect cyclodehydrative ring closure. However, only starting hydrazone **3** was recovered (prolonged reflux in water, ethanol, or 1,2-dichloroethane), or decomposition set in (refluxing 1,2-dichloroethane with protic or Lewis acid).

Upon isolation of **4**, a variety of conditions were explored to generate it selectively. Table 1 summarizes the results. As can be readily seen, there were a number of conditions (entries d, e, f) that yielded solely the 3-phenylpyrimidopyridazinedione (**4**). Interestingly, however, the reaction conditions that afforded cyclized product most rapidly (entry b, 2 equiv NaOAc, H_2O, 1 h) consistently yielded a mixture of both regioisomers, even when varying both the rate and order of addition of the reactants. By careful chromatographic separation of 3- and 4-phenylpyrimidopyridazinedione isomers, we determined that $\delta(H_3) = 8.35$ ppm in DMSO-d_6 for isomer **5**. The pattern of $\delta(H_4) > \delta(H_3)$ is observed for related phenyl substituted compounds (Table 2).

Having established efficient conditions to the 3-phenylpyrimidopyridazinedione (**4**), additional substituted phenyl and selected

Table 2
3- and 4-substituted pyrimido[4,5-*c*]pyridazine-5,7(1*H*,6*H*)-diones synthesized via condensation of 6-(hydrazinyl)uracil **1** with aryl and alkyl glyoxals (**6**)



Entry	R	Conditions[a]	Product (yield)[b]
1a	4-F-Ph	A	**7a** (56%)
1b		B	**7a**:**8a**, 2:3 (55%)
2a	4-Cl-Ph	A	**7b** (22%)
2b		B	**7b**:**8b**, 1:2 (62%)
3a	4-CF_3-Ph	A	**7c** (85%)
3b		B	**7c**:**8c**, 2:5 (43%)
4a	4-MeO-Ph	A	**7d** (75%)
4b		B	**7d**:**8d**, 1:1 (54%)
5a	4-Cl-3-NO_2-Ph	A	**7e** (42%)
5b		B	**7e**:**8e**, 1:1 (51%)
6a	3-F-Ph	A	**7f** (49%)
6b		B	**7f**:**8f**, 1:1 (65%)
7a	*t*-Bu	A	**7g** (19%)[c]
7b		B[d]	**7g** (60%)[c]
8a	Me	A	**7h** (54%)
8b		B	**7h**:**8h**, 3:1 (30%)

[a] A: 1,2-dichloroethane, Δ, 1.5–24 h; B: H_2O, 2 equiv NaOAc, Δ, 0.2–1 h.
[b] Yields represent 1–2 runs for each condition and are not optimized.
[c] Glyoxal from oxidation used directly in cyclization without purification.
[d] Δ, 3 h.

alkyl glyoxal monohydrates were evaluated to explore the scope of the new methodology. Non-commercial glyoxal monohydrates were prepared by oxidation of the corresponding phenyl or alkyl methyl ketone with selenium dioxide according to a standard literature procedure.[11] Each of these was then treated with 6-(hydrazinyl)uracil **1** under the following sets of conditions: (a) refluxing 1,2-dichloroethane, 1.5−24 h and (b) aqueous NaOAc (vide supra). The results are shown in Table 2. In each case, product yields are based on collected precipitate from the reaction mixture and were not optimized. For each example shown, conducting the reaction in 1,2-dichloroethane yielded solely the 3-substituted pyrimidopyridazinedione. For pyruvic aldehyde (Table 2, entry 8a), commercial aqueous reagent was extracted into dichloromethane and the solution dried prior to use to effect a clean reaction to **7h**. Similar biphasic reaction with aqueous reagent resulted in significant co-generation of hydrazone.

In contrast, reactions conducted in aqueous NaOAc yielded a mixture of both regioisomers for all aryl examples examined. Under these conditions both isomers were isolated in approximately equal amounts in many cases (Table 1, entry b, Table 2, 4b, 5b, 6b), but interestingly, if one regioisomer was heavily favoured, it was always the 4-substituted one (Table 2, entries 1b, 2b, 3b). Under the same conditions, when the R group was *t*-butyl (Table 2, entry 7b) only the 3-substituted isomer (**7g**) was formed and was heavily favoured for methyl, reflecting an interesting inversion of regioselectivity between aryl and alkyl glyoxals. To rule out the possibility that product formation proceeds via a hydrazone intermediate, compound **3** was refluxed for 16 h under the NaOAc conditions. Only starting material was recovered.

The ^{1}H NMR spectra for compounds **4a**[2] and **7h**[6] are identical to those described in earlier syntheses, and thus confirm previous structural assignments.

In conclusion, we describe novel and efficient procedures to pyrimido[4,5-*c*]pyridazine-5,7(1*H*,6*H*)-diones. The data argue for a reaction manifold that proceeds by selective nucleophilic attack by the 5-position of the uracil onto the glyoxal aldehyde moiety in nonpolar solvents, whereas for hydroxylic solvents there is a preference for hydrazone formation. Under mildly basic conditions (e.g., aqueous NaOAc), selective C-5 attack is also observed, but now proceeds onto both glyoxal carbonyls with the ratio of 3- and 4-substituted products dependent on electronic and steric factors. In no case is there evidence for ring closure via an intermediate hydrazone. Thus, our methodology allows for the selective incorporation of an aryl or alkyl substituent at the 3-position, representing a direct access to this subclass of pyrimidopyridazinediones. We also report the first entry into 4-monosubstituted isomers and are working to develop conditions that provide this isomer selectively.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.01.007.

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Typical experimental procedures. General procedure for the synthesis of phenyl/alkyl glyoxal monohydrates (**6**) *from phenyl/alkyl methyl ketones*: The phenyl/alkyl methyl ketone (1.5 mmol) was dissolved in 3 mL *p*-dioxane and 120 μL of H_2O. SeO_2 (3.0 mmol, 2 equiv) was added and the mixture was heated at reflux for 24–72 h with consumption of starting material monitored by TLC. The mixture was cooled to 25 °C and filtered through Celite, rinsing with EtOAc. The filtrate was concentrated, dissolved in CH_2Cl_2 and purified by SiO_2 flash chromatography (elution with 0–20% EtOAc in hexanes). *Representative procedure for the synthesis of 3-substituted pyrimido[4,5-c]pyridazine-5,7(1H,6H)-diones. 1,6-Dimethyl-3-phenylpyrimido[4,5-c]pyridazine-5,7(1H,6H)-dione* (**4**): To a refluxing suspension of 6-(hydrazinyl)uracil **1**[8] (85 mg, 0.5 mmol) in 2 mL of 1,2-dichloroethane was added phenylglyoxal monohydrate **2** (84 mg, 0.55 mmol). Additional **2** (10 mg) was added after 3 h and the solution was heated for 3 h more. The orange mixture was concentrated to a solid residue that was triturated in hot EtOH. The solids were collected, washed with EtOH and dried to leave 70 mg (52%) of pure **4** as yellow fibres; mp 260–262 °C (lit[2] mp 250 °C): R_f 0.37 (SiO_2; 95:5 CH_2Cl_2: MeOH); ^{1}H NMR ($CDCl_3$) δ 3.50 (s, 3H), 4.32 (s, 3H), 7.55–7.58 (m, 3H), 7.93–7.95 (m, 2H), 8.69 (s, 1H); ^{1}H NMR (DMSO-d_6) δ 3.26 (s, 3H), 4.17 (s, 3H), 7.55–7.57 (m, 3H), 8.08 (d, J = 3.4 Hz, 2H), 8.72 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 28.25, 43.94, 123.65, 126.79, 128.81, 129.67, 130.85, 133.45, 133.58, 147.65, 155.09, 160.86; MS *m/z* 269.1 (M+H), 291.1 (M+Na). *Representative procedure for synthesis of mixture of 3- and 4-substituted pyrimido[4,5-c]pyridazine-5,7(1H,6H)-diones. 1,6-Dimethyl-3-phenylpyrimido[4,5-c]pyridazine-5,7(1H,6H)-dione* (**4**) *and 4-phenyl isomer* (**5**): To a refluxing mixture of 6-(hydrazinyl)uracil **1**[8] (85 mg, 0.5 mmol), NaOAc (82 mg, 1 mmol), and 4 mL of distilled H_2O was added phenylglyoxal monohydrate (84 mg, 0.55 mmol) in a single charge. Yellow precipitate began to form immediately. After heating for 1 h, the supension was maintained at 25 °C for 1 h. The solids were collected, washed with H_2O, and dried to leave 102 mg (76%) of ~1:1 mixture of **4**:**5** by ^{1}H NMR. TLC (SiO_2; 97:3 CH_2Cl_2/MeOH);) showed two partially overlapping spots, R_f 0.24. A small sample of the mixture was purified by preparative SiO_2 thin layer chromatography, eluting 2 × with 98:2 CH_2Cl_2/MeOH), to provide pure **5**; mp >205 °C (dec): ^{1}H NMR ($CDCl_3$) δ 3.38 (s, 3H), 4.25 (s, 3H), 7.42–7.44 (m, 3H), 7.54–7.57 (m, 2H), 8.10 (s, 1H); ^{1}H NMR (DMSO-d_6) δ 3.14 (s, 3H), 4.07 (s, 3H), 7.48–7.50 (m, 5H), 8.35 (s, 1H); MS *m/z* 269.1 (M+H), 291.1 (M+Na).