# One-Pot Synthesis of 5-Hydroxy-4*H*-1,3-thiazin-4-ones: Structure Revision, Synthesis, and NMR Shift Dependence of Thiasporine A

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Ⓢ *Supporting Information*

ABSTRACT: An annulation of arylthioamides with 3-bromopyruvic acid chloride to 5-hydroxy-4*H*-1,3-thiazin-4-ones has been developed. The initial condensation affords two regioisomeric thiazolinone intermediates in a temperature-dependent manner. The synthesis of the 2-aminophenyl-thiazinone derivative led to the revision of the previously proposed structure of thiasporine A. Synthesis of the revised structure and NMR analysis revealed that thiasporine A had been isolated as a carboxylate.
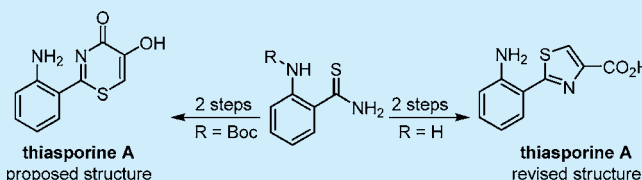
In 2015, the MacMillan laboratory reported[1] the isolation of thiasporines A−C from a marine-derived *Actinomycetospora chlora* (Figure 1). While thiasporines B and C had been isolated
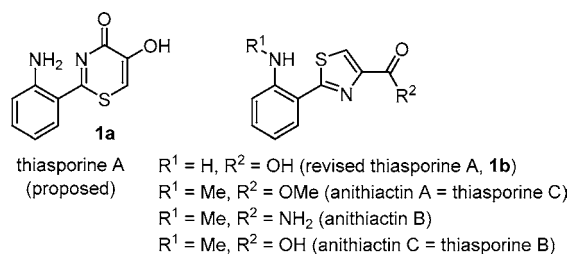

Figure 1. Structures of the thiasporines and anithiactins.

previously as anithiactins by Kang, Nam and co-workers,[2] thiasporine A (**1a**) appeared to be clearly different. Its structure was assigned to contain a 5-hydroxy-4*H*-1,3-thiazin-4-one moiety[3] which was the first example of such a ring system in a natural product. Motivated by the unusual heterocycle and its activity against the nonsmall-cell lung cancer cell line H2122 (IC_{50} 5.4 μM),[4,5] the Christmann laboratory completed a synthesis of 5-hydroxy-4*H*-1,3-thiazin-4-ones from arylthioamides. Comparison of the ^{1}H and ^{13}C NMR spectra for isolated thiasporine A and synthetic **1a** indicated a problem with its structural assignment. Herein, we report the synthesis of the originally proposed structure for thiasporine A (**1a**) and the determination and synthesis of the revised structure (**1b**). These joint efforts underscore the indispensable role of chemical synthesis for validating structural assignments.

Our annulation strategy for the synthesis of the proposed structure of thiasporine A (**1a**) was focused on using a thioamide and a pyruvate derivative as readily available coupling partners. In the classical Hantzsch thiazole synthesis, α-halo ketones (**2**) and thioamides are joined (Scheme 1a). In order to

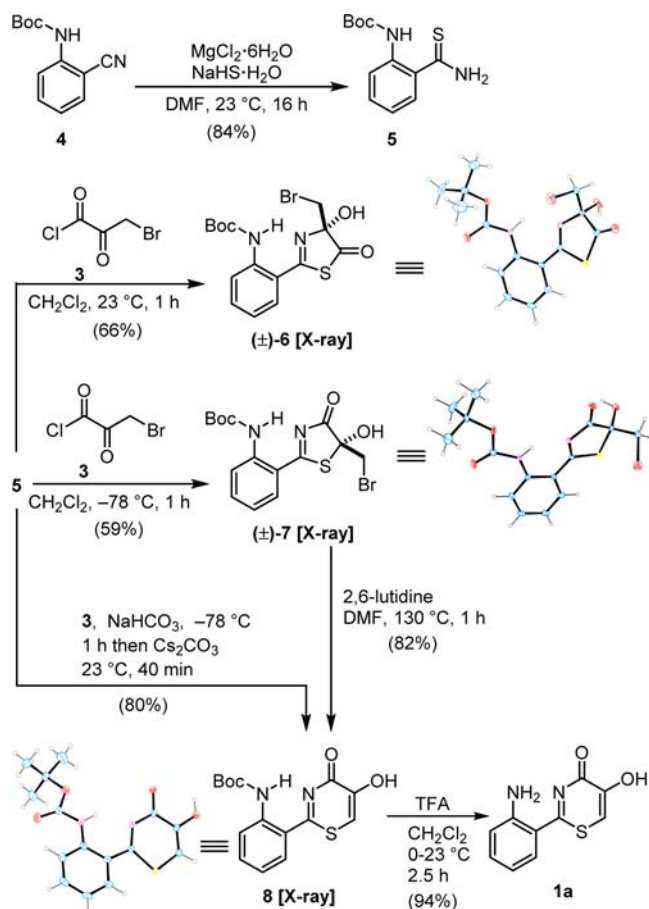**Scheme 1. Reactions between Thioamides and Pyruvates**
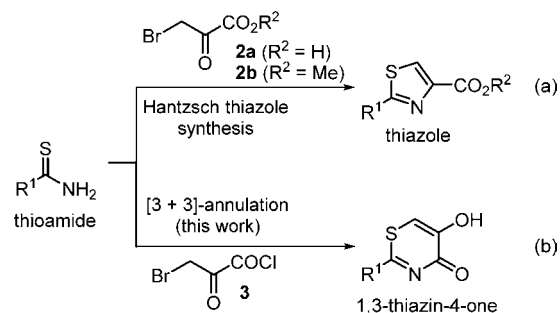
outpace competing thiazole formation in favor of the desired thiazinones (Scheme 1b), we deemed it necessary for the anticipated [3 + 3]-annulation to activate C1 in pyruvate (e.g., as acyl chloride **3**). While preparations[6−10] and reactions[11,12] of 4*H*-1,3-thiazin-4-ones have been reported, a direct synthesis from arylthioamides and pyruvates is not known to the best of our knowledge.

The synthesis of **1a** commenced with the preparation of thioamide **5** from commercially available Boc-protected amino-benzonitrile **4** using sodium thiolate in the presence of magnesium chloride (Scheme 2).[13] The arylamino group was protected in order to inhibit amide formation with the acyl halide. Treatment of **5** with freshly prepared 3-bromopyruvic acid chloride **3** in dichloromethane at room temperature led, quite unexpectedly, to *S*-acylation and hemiaminal formation to subsequently yield 1,3-thiazolin-5-one (±)-**6** in 66% yield.[14] Lowering the temperature to −78 °C completely reversed the regioselectivity in favor of the desired *N*-acylation to furnish 1,3-thiazolin-4-one (±)-**7**. This material could be isolated, but

Scheme 2. Synthesis of 1a
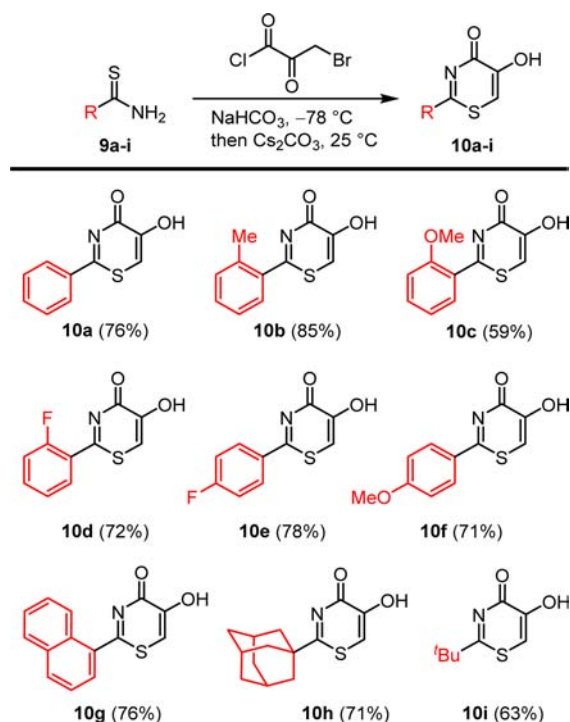

Scheme 3. Substrate Scope of the One-Pot Synthesis of 5-Hydroxy-4H-1,3-thiazin-4-ones 10[a]

[a]Yields refer to isolated material. For detailed reaction conditions, see the Supporting Information.

the hypothesis that the carboxylate ion might cause significant changes to the ^{13}C shifts (Scheme 4).

Scheme 4. Synthesis of 1b

partial conversion to 1,3-thiazinone-4-one **8** was observed during warming. Heating of **7** in DMF in the presence of 2,6-lutidine afforded **8** in good yield. The structures of intermediates **6**−**8** were confirmed by X-ray crystallographic analysis.[15] Extensive experimentation revealed conditions that allowed telescoping the latter two transformations into an efficient one-pot process.[16] Accordingly, thiazinone **8** could be obtained in 80% yield from **5**. In the final step, the Boc-protecting group was removed with trifluoroacetic acid in dichloromethane to give target compound **1a** in 94% yield.
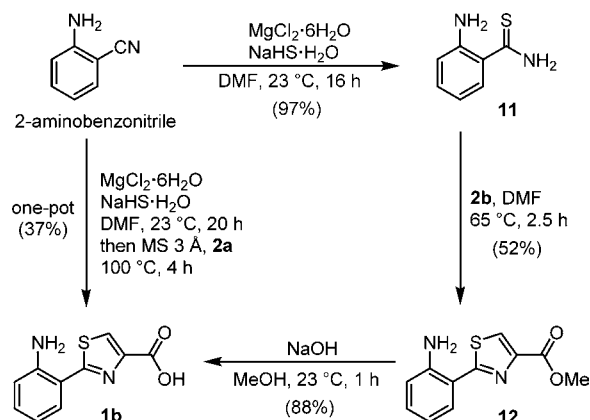
As shown in Scheme 3, the above-described one-pot, two-step synthesis of 4H-1,3-thiazin-4-ones **10a**−**g** was demonstrated to be tolerant of a small set of arylthioamides **9** containing both electron-withdrawing and -donating substituents. Moreover, thioamides with bulky alkyl residues were tolerated and provided the corresponding thiazinone products **10h**−**i** in good yield.

Comparison of the ^{1}H and ^{13}C NMR data of synthetic **1a** and isolated thiasporine A showed clear differences in the chemical shifts on the heterocyclic moiety (Figure S1). This discrepancy coupled with the coisolation of thiazole-4-carboxylic acid containing thiasporines B and C led us to reconsider the structure of thiasporine A. The original assignment of the 5-hydroxy-4H-1,3-thiazin-4-one moiety was in part motivated by the significant differences of the ^{13}C chemical shifts compared to the thiazole-4-carboxylic acid moieties found in thiasporine B and C. Thus, we decided to carry out a synthesis of the thiazole-4-carboxylic acid **1b** with

By adapting known[2,13] protocols, 2-aminobenzonitrile was treated with sodium hydrosulfide in the presence of magnesium chloride to give thioamide **11**, which was subsequently condensed with methyl bromopyruvate **2b**. Comparison of the ^{1}H NMR of synthetic **12** with the ^{1}H NMR of methylated thiasporine A showed they were identical (Figure S2), suggesting that thiasporine A contains the thiazole-4-carboxylic acid moiety. Hydrolysis of **12** with NaOH gave **1b** in an 88% yield. Anticipating future SAR studies, we also demonstrated that it is possible to streamline the synthesis of **1b** to a one-pot operation albeit with a reduced yield of 37%. Surprisingly, comparison of the NMR data for synthetic **1b** with isolated
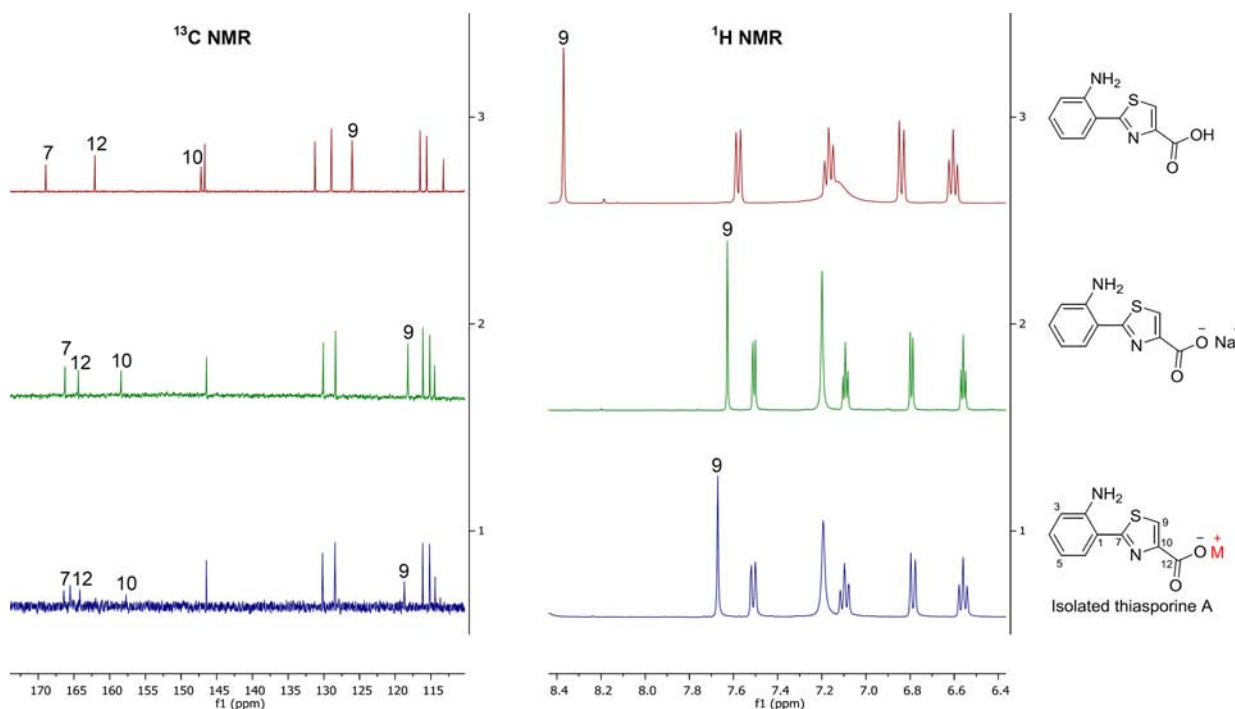
Figure 2. Comparison of the ^{1}H and ^{13}C NMR of synthetic **1b** and its sodium salt with that of isolated thiasporine A in DMSO-d_6.

thiasporine A revealed significant differences of up to 9.5 ppm in the ^{13}C NMR spectrum of the heteroaromatic region (Figure 2). In particular, carbons C-7, C-9, C-10, and C-12 suffer substantial changes in the chemical shift (see the Supporting Information for a titration experiment and the effect of different counterions). This result prompted us to consider the possibility that thiasporine A was isolated as a carboxylate. In order to probe this hypothesis, the salts of **1b** were prepared. As shown in Figure 2, the ^{1}H and ^{13}C NMR spectra of the sodium salt compare nicely with isolated thiasporine A. The dramatic chemical shift differences in the carboxylate also explain the difference between isolated thiasporine A and thiasporines B and C. In 4-thiazolecarboxylic acid (**A**), mesomeric representations **B** and **C** can be used to reflect a relative decrease in electron density (deshielding) at C-9 and increased electron density at C-10 (Figure 3). In the case of the corresponding carboxylate **A′**, mesomeric forms **B′** and **C′** become less favorable (compared to **B** and **C**) due to electron repulsion. Similar shift dependences have been reported with coumaric acids and the corresponding alkali metal coumarates.[17]
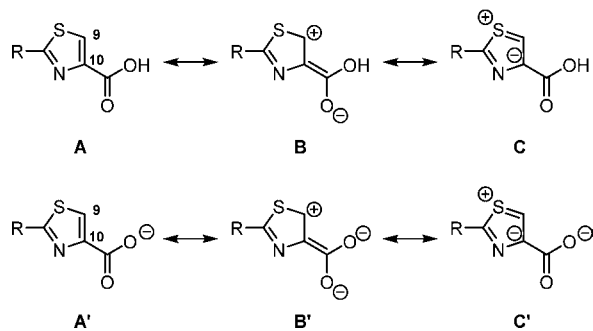

Figure 3. Mesomeric forms of a 4-thiazolecarboxylic acid (**A**) and its corresponding carboxylate (**A′**).

In summary, we have revised the structure of thiasporine A from **1a** to **1b** by chemical synthesis using a newly developed [3 + 3]-annulation between 3-bromopyruvic acid chloride and arylthioamides. The unusual NMR shifts in isolated thiasporine A were attributed to its isolation as a carboxylate salt as shown by comparison with the NMR spectra of the free carboxylic acid.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b01166.

Crystallographic data (CIF, CIF, CIF)
Procedures and spectroscopic data (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

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(15) CCDC 1473774 (**6**), 1473776 (**7**), and 1473775 (**8**) contain the supplementary crystallographic data for this publication. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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