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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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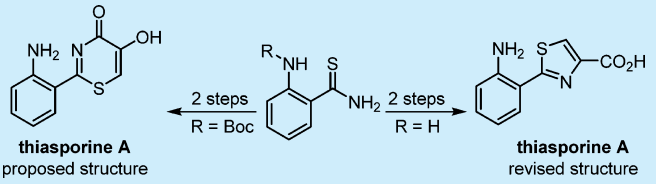
Supporting Information

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 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.

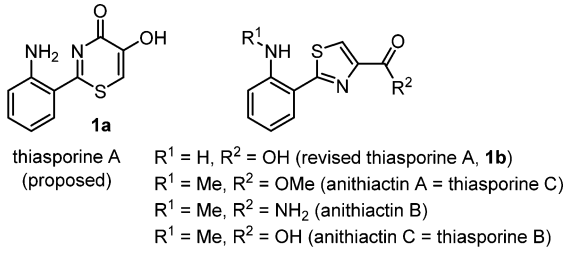
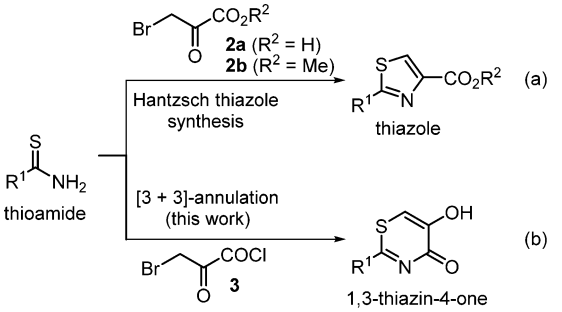


Figure 1. Structures of the thiasporines and anithiactins.

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 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.

Scheme 1. Reactions between Thioamides and Pyruvates



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

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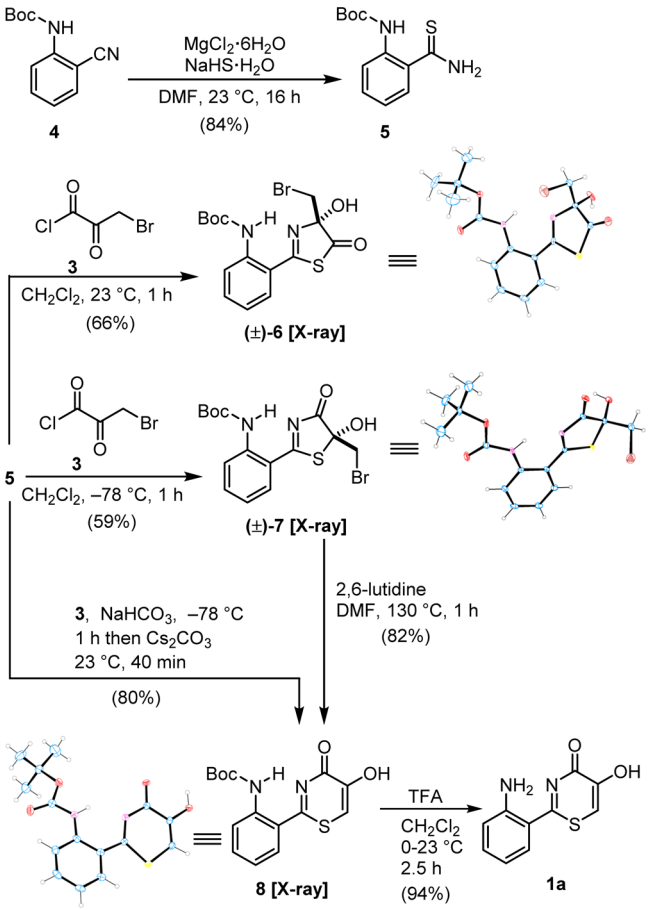
Scheme 2. Synthesis of 1a

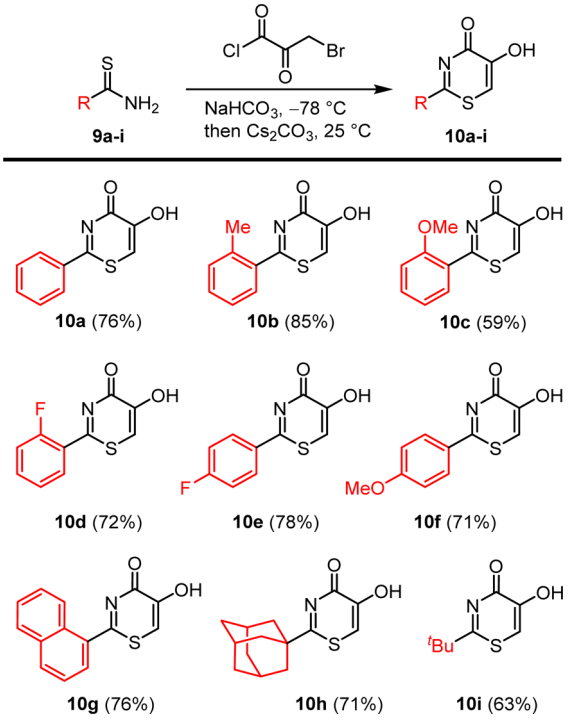


partial conversion to 1,3-thiazinone-4-one **8** was observed during warming. Heating of **7** in DMF in the presence of 2,6-lutidine afforded **8** in good yield. The structures of intermediates **6**–**8** were confirmed by X-ray crystallographic analysis.[15] Extensive experimentation revealed conditions 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 4*H*-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-4*H*-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

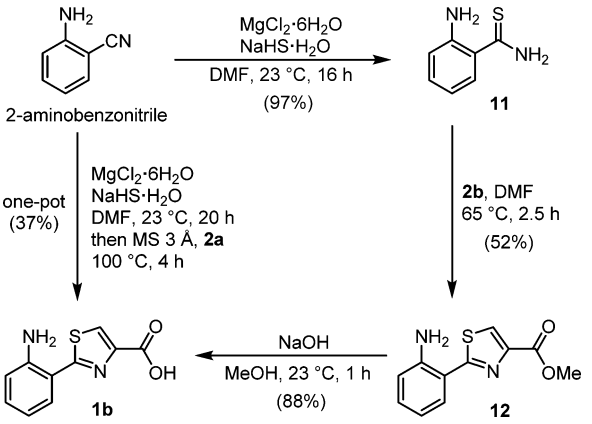
Scheme 3. Substrate Scope of the One-Pot Synthesis of 5-Hydroxy-4*H*-1,3-thiazin-4-ones 10[a]



[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



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

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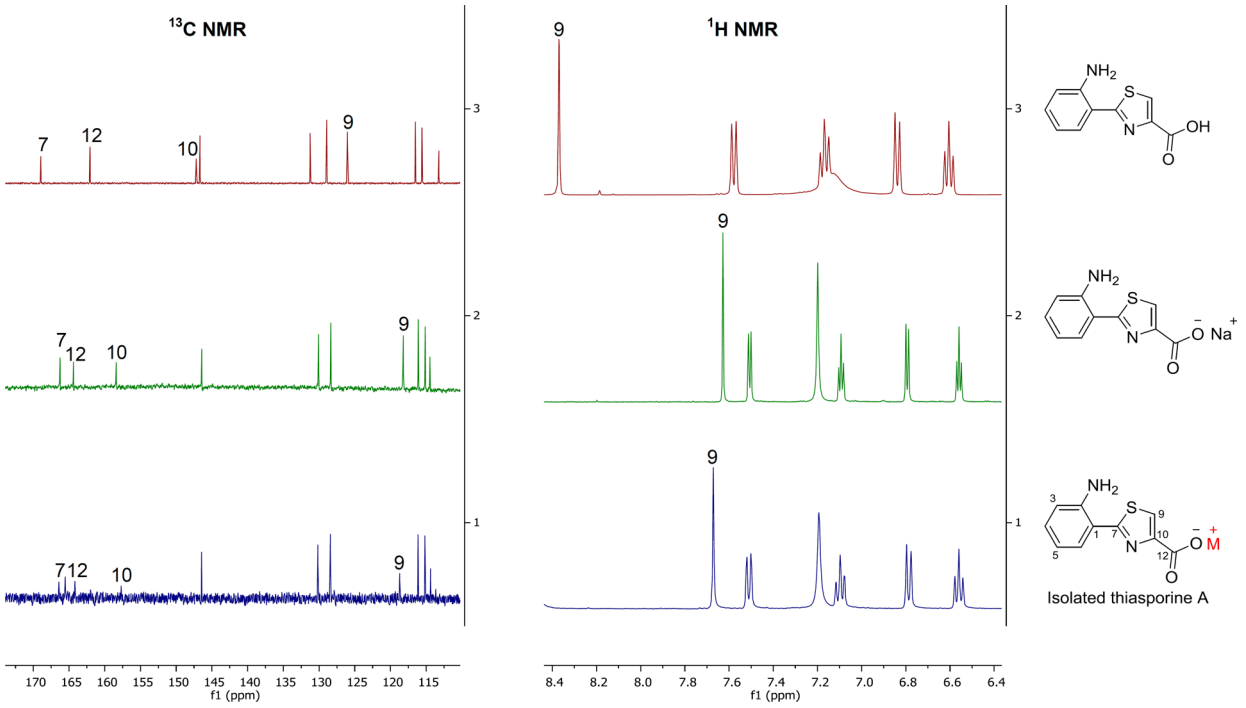

Figure 2. Comparison of the ^{1}H and ^{13}C NMR of synthetic **1b** and its sodium salt with that of isolated thiasporine A in DMSO-d_6.

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]

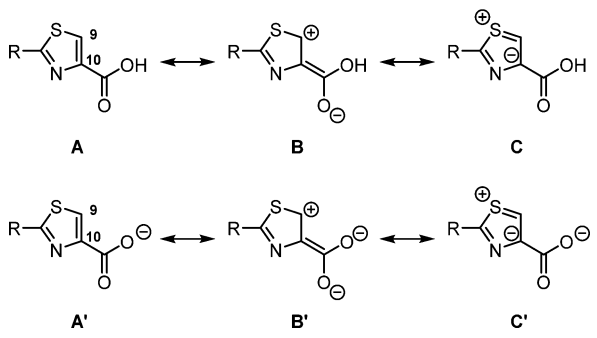

Figure 3. Mesomeric forms of a 4-thiazolecarboxylic acid (**A**) and its corresponding carboxylate (**A′**).

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.

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■ REFERENCES

(1) Fu, P.; MacMillan, J. B. *J. Nat. Prod.* **2015**, *78*, 548−551.
(2) Kim, H.; Yang, I.; Patil, R. S.; Kang, S.; Lee, J.; Choi, H.; Kim, M.-S.; Nam, S.-J.; Kang, H. *J. Nat. Prod.* **2014**, *77*, 2716−2719.
(3) Ryabukhin, Y. I.; Korzhavina, O. B.; Suzdalev, K. F. Chemistry of 1,3-Thiazin-4-ones and Their Derivatives. In *Advances in Heterocyclic*

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Org. Lett. XXXX, XXX, XXX−XXX

Chemistry; Alan, R. K., Ed.; Academic Press: 1996; Vol. *66*, pp 131−191.
(4) For the synthesis and biological evaluations of anithiactins/thiasporines, see: Lamb, R. A.; Badart, M. P.; Swaney, B. E.; Gai, S.; Baird, S. K.; Hawkins, B. C. *Aust. J. Chem.* **2015**, *68*, 1829−1833.
(5) Lee, H. W.; Jung, W. K.; Kim, H. J.; Jeong, Y. S.; Nam, S.-J.; Kang, H.; Kim, H. *J. Microbiol. Biotechnol.* **2015**, *25*, 1425−1428.
(6) White, E. H.; Suzuki, N.; Miano, J. D. *J. Org. Chem.* **1978**, *43*, 2366−2369.
(7) Goerdeler, J.; Tiedt, M.-L.; Nandi, K. *Chem. Ber.* **1981**, *114*, 2713−2722.
(8) Yamamoto, Y.; Ohnishi, S.; Azuma, Y. *Chem. Pharm. Bull.* **1983**, *31*, 1929−1935.
(9) Yokoyama, M.; Sato, K.; Tateno, H.; Hatanaka, H. *J. Chem. Soc., Perkin Trans. 1* **1987**, 623−628.
(10) Danilkina, N. A.; Mikhailov, L. E.; Ivin, B. A. *Russ. J. Org. Chem.* **2006**, *42*, 783−814.
(11) Abouelfida, A.; Roze, J.-C.; Et, J.-P. P.; Jubault, M. *Phosphorus, Sulfur Silicon Relat. Elem.* **1990**, *54*, 123−134.
(12) Yamamoto, Y.; Ohnishi, S.; Moroi, R.; Yoshida, A. *J. Chem. Soc., Chem. Commun.* **1983**, 56−58.
(13) Oschatz, S.; Brunzel, T.; Wu, X.-F.; Langer, P. *Org. Biomol. Chem.* **2015**, *13*, 1150−1158.
(14) Reactions of α-haloacyl halides with thioamides gave exclusive *N*-acylation: Okawara, T.; Kashihara, H.; Furukawa, M. *Chem. Pharm. Bull.* **1985**, *33*, 3479−3483.
(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.
(16) Vaxelaire, C.; Winter, P.; Christmann, M. *Angew. Chem., Int. Ed.* **2011**, *50*, 3605−3607.
(17) Kowczyk-Sadowy, M.; Świsłocka, R.; Lewandowska, H.; Piekut, J.; Lewandowski, W. *Molecules* **2015**, *20*, 3146−3169.

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