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Convenient synthesis of N1-substituted orotic acid derivatives

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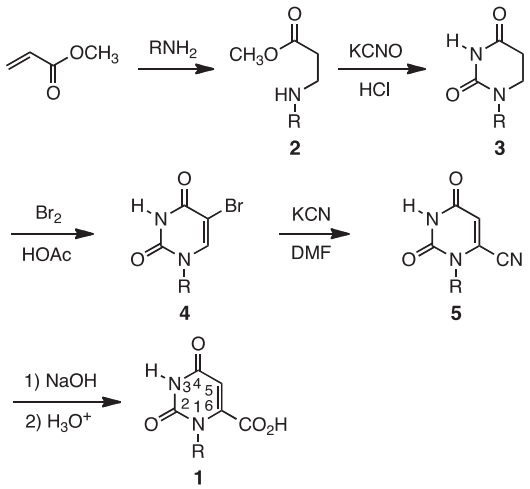
ABSTRACT

A convenient and efficient method for the synthesis of N1-substituted orotic acid derivatives is reported. The synthetic route utilizes substituted maleimide as synthetic intermediate and takes only four simple steps from readily available starting materials. As a result, orotic acid derivatives with various alkyl and aromatic groups at N1 can be readily synthesized.

Orotic acid and its analogs have been investigated as chemical models for the reaction catalyzed by orotidine-5′-monophosphate decarboxylase (ODCase).[1–12] We are interested in understanding the effect of hydrogen bonds to the pyrimidine moiety on the rate of decarboxylation. This investigation requires N1-substituted orotic acid derivatives (structure **1** in Scheme 1) as substrates. Unfortunately N1-substituted orotic acid cannot be prepared from the alkylation of orotic acid because N-3 is the more reactive site.[13] The reported synthesis of N1-substituted orotic acid derivatives is time-consuming and low-yielding (Scheme 1, the combined yield for the synthesis of 1-cyclohexylorotic acid is about 15%).[11,14–18] Furthermore, we have found that the purification of the final orotic acids can be difficult sometimes. In this Letter, we report a convenient and efficient synthesis of N1-substituted orotic acid derivatives from readily available starting material.

Unsubstituted orotic acid (**1**, R = H) has been prepared from glutamic acid through the intermediate hydantoin **6** (R = H, Scheme 2), which is converted to orotic acid **1** (R = H) upon treatment with hydroxide.[19] Unfortunately, N-substituted **6** (prepared from the aldol condensation of N1-substituted hydantoin with glyoxylic acid)[20] is very stable and has been reported to resist rearrangement to orotic acid under various conditions.[21]

On the other hand, *N*-carboethoxymaleimide **7** has been reported to rearrange to N1-substituted orotic acid upon treatment with hydroxide as shown in Scheme 3, although the yields were low for non-phenyl substituents.[22] We have thus designed a convenient synthetic method for the substituted maleimide **7** from the commercially available maleimide **8**. Bromination of **8** gave the 2,3-dibromosuccinimide mixed with 2-bromomaleimide.[23] Separation of the two products was not necessary because both products eventually yielded the aminosubstituted maleimide **9** upon treatment with alkylamine or arylamine.[23] Although methanol and *N*-methylpyrrolidinone have been employed for similar



Scheme 1.

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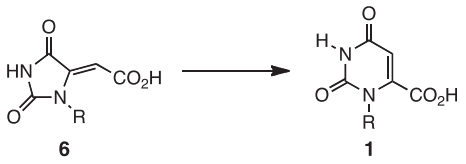
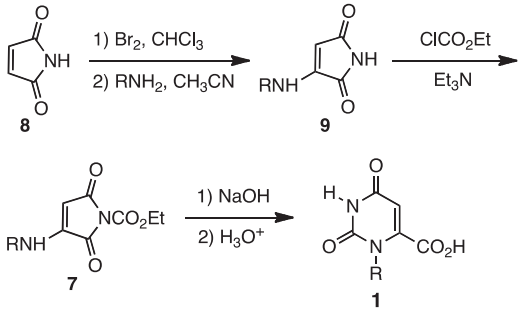


Scheme 2.



Scheme 3.

Table 1
Yields for the synthesis of orotic acid **1** from maleimide **8**

Entry	Substituents (R=)	Yield[a] (**8**–**9**) (%)	Yield[a] (**9**–**1**) (%)
a	Cyclohexyl	83	71
b	*n*-Butyl	78	68
c	Phenyl	78	90
d	Neopentyl	79	51
e	Allyl	100	70
f	Benzyl	90	69

[a] Isolated yield.

reactions,[24,25] acetonitrile was found to be the best solvent. The reactions were conveniently carried out at room temperature overnight. Treatment of **9** with ethyl chloroformate gave the desired synthetic intermediate **7**, which did not require further purification and was readily converted to N1-substituted orotic acid **1** via an improved procedure.[22,26]

The reaction has been successfully carried out with various alkylamine or arylamine substrates. This method thus allows the successful synthesis of orotic acid derivatives with various substituents at N1 in good yield as reported in Table 1. The previously reported synthetic route as seen in Scheme 1 is not only lengthy but also limited to non-allylic and non-benzylic alkyl groups. In the third step in Scheme 1 (the bromination of dihydrouracil **3** with Br_2/HOAc), bromination occurred readily at unwanted positions when substrates substituted with allylic, benzylic, or aromatic groups were utilized. The conversion from dihydrouracil **3** to bromouracil **4** was thus unsuccessful for substrates with these groups. The current method, however, tolerates a diverse group of substituents.

In summary, the new method allows the convenient synthesis of N1-substituted orotic acid derivatives from readily available starting material in good yield. The method works well for substrates with a variety of substituents such as aromatic or alkyl (including allylic or benzylic) groups. It should be pointed out that this synthetic route also involves sequential incorporation of nitrogen atoms to the pyrimidine structure and thus should allow the incorporation of a single ^{15}N label at N-1. The method represents a significant improvement from the previously reported synthetic route.

Acknowledgments

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26. *Experimental details:* All reagents were obtained from commercial sources and used without further purification. Typical experimental procedures are described below using 1-cyclohexylorotic acid (**1a**) as an example.

*Synthesis of 3-cyclohexylmaleimide (**9a**):* To a solution of maleimide (2.0 g) in 15 mL of chloroform was added a solution of bromine (2.5 mL) in 15 mL of chloroform dropwise. Upon refluxing for 2 h, the reaction mixture was evaporated to dryness. The off-white solid (5.3 g) obtained was identified by NMR to be a mixture of *cis*- and *trans*-2,3-dibromosuccinimide as well as 3-bromomaleimide. The crude product mixture was used directly in the next step without further purification.

To a solution of the crude mixture (1.0 g, 4 mmol) in 2 mL of acetonitrile was added a solution of cyclohexylamine (1.0 g, 12 mmol) in 12 mL of acetonitrile and the solution was stirred at room temperature overnight. The precipitate was filtered and washed with methylene chloride. The mother liquor was concentrated to dryness and purified by column chromatography to yield 3-cyclohexylaminomaleimide (**9a**) as a yellow solid (0.6 g, 83% combined yield).

*Synthesis of 1-cyclohexylorotic acid (**1a**):* Maleimide **9a** (0.30 g, 1.5 mmol) was dissolved in 15 ml anhydrous acetone. Ethyl chloroformate (0.26 g, 2.4 mmol) and triethylamine (0.24 g, 2.4 mmol) were added dropwise simultaneously with stirring. After completion of the addition, the mixture was stirred for 45 min and then refluxed for 45 min. Upon standing overnight the reaction mixture was filtered to remove the precipitated triethylammonium salt and washed with methylene chloride. The mother liquor was evaporated to dryness to give the crude 1-ethoxycarbonyl-3-cyclohexylaminomaleimide **7a**, which was suspended in 8.0 ml 1 M aqueous KOH and heated at 65 °C for 2 h. The reaction mixture was acidified with 6 N HCl and concentrated to about a third of the original volume. The resulting off-white precipitate was filtered, washed with cold water and ether, and recrystallized from water or water/ethanol to give 1-cyclohexylorotic acid **1a** as a white solid (0.26 g, 71%).