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ARTICLE TYPE

Graphical Abstract

A convenient, highly selective and eco-friendly N-Boc protection of pyrimidine nucleobases under microwave irradiation

Maxime Bessières, Vincent Roy, Luigi A. Agrofoglio*

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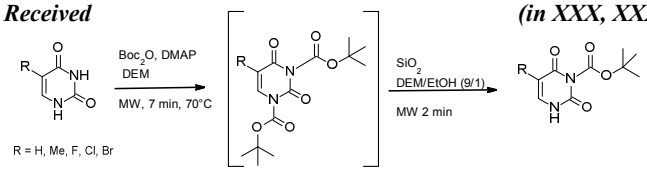

A novel and practical microwave-assisted, highly selective N3-Boc protection of pyrimidine nucleobases in eco-friendly DEM/EtOH solvent, under mild conditions and moderate to high yield is described.

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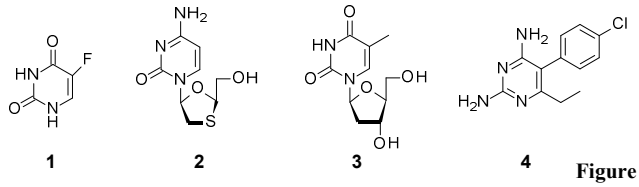
Protected pyrimidine nucleobases are of major importance as intermediates in the synthesis of nucleoside analogues and molecules with biological interests. We described herein a novel practical microwave-assisted *N*-Boc protection of pyrimidine nucleobases under mild conditions using silica gel, avoiding treatment steps, and in increased yield.

Pyrimidine systems are found in many drugs interacting with the synthesis and functions of nucleic acids such as the antitumor fluorouridine[1] (**1**), the anti-HIV drug lamivudine[2] (**2**) or the anti-HBV drug telbivudine[3] (**3**), or the antimalarial drug pyrimethamine[4] (**4**), (Figure 1), to only quote some of them.

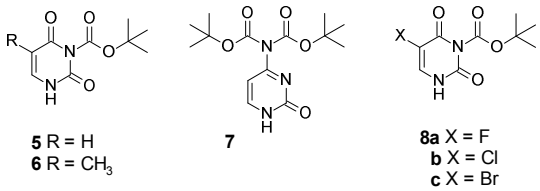


1 **2** **3** **4**

Figure 1. Chemotherapeutics containing a pyrimidine moiety

As chemical modifications of nucleobases are of paramount importance in the development of new drugs,[5] their syntheses have been largely described.[6] Focusing on the base moiety, a key step is the choice of the appropriate protecting groups for the N1 and N3 of pyrimidines which have to meet several requirements such as: a differential protection at N1 and N3, a selective deprotection, and a removal under mild reaction conditions. There is an abundant literature relating to the protection of amino groups, such as by 9-fluorenyl-methoxycarbonyl (FMOC),[7] *tert*-butyl carbamates (Boc), benzyl carbamate, benzamide, acetamide,[8] phtalimides,[9] triphenyl methyl amide[10] and tosylamide.[11]

Among these groups, one of the most used is the Boc; it's stable towards most nucleophiles and bases[12] and it can be removed cleanly and selectively under neutral conditions.[13] This is of particular interest when working with biolabile groups such as found in nucleoside prodrugs.[14] Surprisingly, only two teams have reported the synthesis of N-Boc-protected pyrimidines under basic conditions and in two steps. Gothelf et al.[15] have prepared the N3-Boc-protected thymine (**6**) through the formation of N1,N3-di-Boc protected derivative and subsequent selective deprotection of N1-Boc group with K_2CO_3 in dioxane, in only 31% overall yield (Figure 2). Porcheddhu et al.[16] have described the synthesis of N4,N4-di-Boc cytosine (**7**) in two steps in moderate 60% yield, *via* the fully Boc protected cytosine which was then treated with aq. $NaHCO_3$ in MeOH at reflux. However, in spite of their potential utility, these methods suffer from some drawbacks such (a) a long reaction time implied by the first step, (b) unsatisfactory yields of the deprotection step and (c) cumbersome product isolation procedures. Thus, we present herein, a greener practical protocol to selectively synthetize N1-free,N3-Boc protected pyrimidine nucleobases (**5-7**, **8a-c**) under milder conditions, shorter reaction time and in moderate to quantitatively yields.



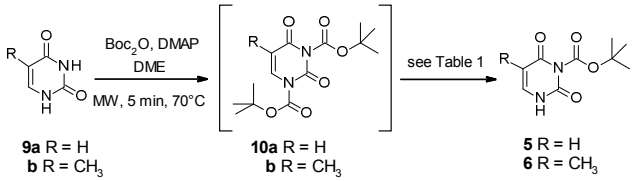
5 R = H
6 R = CH_3

7

8a X = F
b X = Cl
c X = Br

Figure 2. Structure of monoprotected pyrimidic nucleobases

The general strategy involves two steps: (1) the use of microwave irradiation to reach quantitatively the fully protected pyrimidines (**10a,b**) and (2) the subsequent selective N1-deprotection by treatment with SiO_2[17] in diethoxymethane/ethanol (9:1) as an eco-friendly surrogate to CH_2Cl_2/MeOH, to desired compounds **5** and **6**, respectively (Scheme 1). It is important to quote that microwave activation, which ensures shorter reaction time, cleaner reactions and which has been applied to nucleosides and their precursors,[18] has never been reported for either the Boc-protection or deprotection of pyrimidines.



9a R = H
b R = CH_3

10a R = H
b R = CH_3

5 R = H
6 R = CH_3

Scheme 1. General approach to N1 free, N3-Boc-pyrimidines.

Thus, starting with uracil **9a**, we applied the microwave assisted, solvent-free and catalyst-free procedure reported by Dighe et al.[19] Unfortunately, in our hands, probably due to the insolubility of

uracil derivatives in Boc_2O, the expected compounds were not isolated. We moved then to a solution-phase preparation of N1,N3-di-Boc uracil (**10a**) under microwave irradiation in dry diethoxymethane (DEM) as a replacement of THF, with an excess of Boc_2O, in presence of 0.35 equivalents of 4-*N,N*-(dimethylamino)pyridine (DMAP) at 70 °C. It is interesting to quote that DEM is an attractive alternative to conventional solvents as it is an eco-responsible solvent, with unique properties,[20] which make it a green industrial solvent.

After evaporation of all volatiles, compound **9a** was almost quantitatively converted to **10a** (based on TLC) and was engaged in the next step without the need for further purification. Table 1 summarizes the different conditions used for the N1 regioselective deprotection of **10a** to **5**.

Table 1: Preparation of N3-tert-butylcarbonyluracil (**5**) by selective deprotection of **10a** with SiO_2.

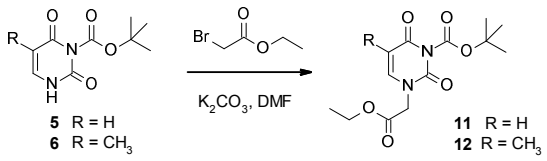
Entry	Solvent	Temperature	Reaction time	Yield (%)
1[a]	DCM	RT	5h	43
2[a]	DCM/MeOH (9/1)	RT	1h30	93
3	DCM/MeOH (9/1)	RT	12h	77
4[a]	DCM/MeOH (9/1)	60°C	1h	96
5[a]	DCM/MeOH (9/1)	MW, 70°C	2 min	97

[a] Reaction performed with SiO_2 (60% w/w)

Based on the work of Zhang *et al.,*[17] the N1,N3-di-(Boc) uracil (**10a**) was reacted with SiO_2 in dichloromethane (entry 1). To avoid the full deprotection, the reaction was performed at room temperature and monitored by TLC. After 5 hours, the reaction was stopped by simple evaporation of volatiles, affording a solid deposit for flash chromatography. Under this condition, **5** was obtained in 44% yield. When increasing the polarity to CH_2Cl_2/MeOH (9/1), the desired compound **5** was afforded in 90 minutes at room temperature, in 93% yield (entry 2). The benefits of SiO_2 was confirmed (entry 3) observing the dramatically decreased yield from 93% to 77% yield obtained after 12 hours whereas the activation of the reaction over classical heating increases the yield and reduces the reaction time (entry 4). With our interest in shorter reaction time, the previous conditions were optimized under microwave activation to afford quantitatively the desired compound (entry 5).

Following the same procedure, N1 protected thymine (**6**) was obtained in good overall yields from **9b**. This straight method offers thus a direct solid deposit for flash chromatography after evaporation of all volatiles, avoiding any pre-treatment.

To investigate the final regioselectivity of the Boc derivatives (N1 *versus* N3), the N1-alkylation was performed and the selectivity fully determined by NMR. For instance, compound **6** was derivatized to **12** with ethyl bromoacetate in the presence of K_2CO_3) (Scheme 2).



Scheme 2. Derivation at N1 position for NMR study

Structure of **12** was determined by HMBC experiment, confirming the regioselectivity the deprotection step (Figure 3).

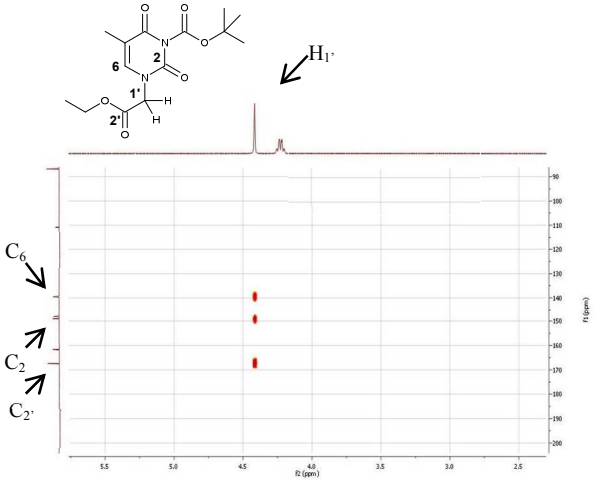
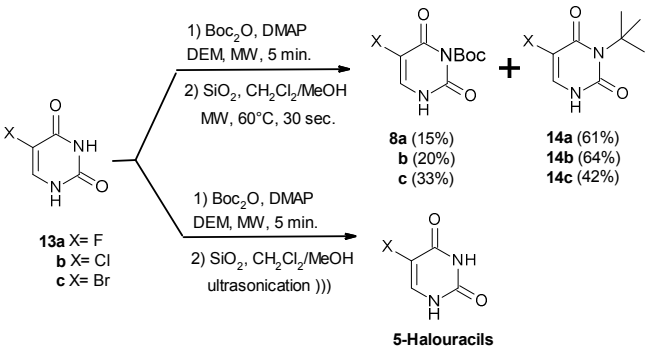


Figure 3. HMBC NMR analysis of **12**

Then we turned our attention to C5-halopyrimidines which are of great interest as useful synthetic building blocks for further C5 modifications. Surprisingly when applying this two-steps procedure to the C5-halogenopyrimidines (**13a-c**), we observed the minor formation of the expected N3-Boc uracils (**8a-c**) concomitant with the major N3-alkylation with a *tert*-butyl group (**14a-c**, respectively) (Scheme 3).

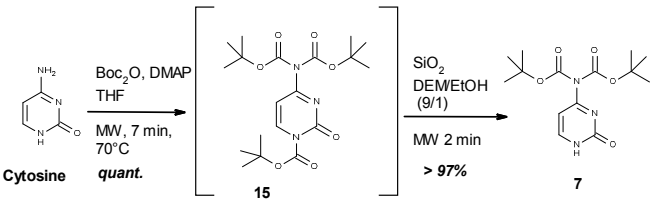


Scheme 3. Deprotection of bis-Boc halogenated uracils

To explain the formation of **14a-c**, we hypothesized that : (1) under our conditions (e.g. slightly acidic), the σ-electron withdrawing effect of the C5-halogens (fluoro, chloro, and

bromo) can help to release the Boc at N3, and (2) the more nucleophilic N3 obtained can react with the released *iso*butene under microwave heating in closed vials. When the deprotection step was performed under ultrasounds, due to their "degassing effect", only the unprotected C5-halogenated uracils were isolated in quantitatively yields.
In order to be as eco-friendly as possible, we have extended the investigation of the DEM/EtOH solvent system, phasing out the dichloromethane and in a less extend, the methanol. Under our optimized conditions (e.g., slightly acidic SiO_2 under microwaves), DEM is an attractive replacement solvent for DCM. Compared to methanol, ethanol is produced by factory fermentation of food crops and it's more ecological than methanol.

Thus, the final optimized conditions were SiO_2 60% w/w in DEM/EtOH (9/1) under microwaves irradiation. Applied to cytosine, the N4,N4-di-Boc cytosine **7** was reached through **15** in 2 minutes under microwaves at 70 °C with more than 97% yield (Scheme 4).



Scheme 4. Synthesis of derivative **7** under eco-friendly conditions

In summary, we have accomplished a convenient, highly selective and eco-friendly N-Boc protection of some pyrimidine nucleobases (**5-7**, **8a-c**) under microwave irradiation. The mild and heterogeneous conditions are provided by SiO_2, acid enough to support the deprotection of carbamates. The microwave irradiation has proved to be the most efficient synthesis route, giving the desired compounds with quantitative (for **5-7**) or moderate (for **8a-c**) yields, in short reaction times. The eco-friendly diethoxymethane and ethanol were chosen as a greener alternative to more toxic dichloromethane and methanol, respectively. These advantages make from these reactions some powerful ecological alternatives, optimizing the synthesis of Boc-thymine and *bis*-Boc-cytosine and creating a synthetic way for Boc-uracil. This environmentally friendly approach represents a promising way to synthesize these necessary building blocks for the synthesis of nucleosides, of utmost importance in medicinal chemistry.

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Notes and references

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† Electronic Supplementary Information (ESI) available: [general procedure and ^{1}H, ^{13}C NMR spectra are provided]. See DOI: 10.1039/b000000x/

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