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Microwave-Promoted "One-Pot" Synthesis of 4-Nitrobenzylthioinosine Analogues Using Thiourea as a Sulfur Precursor

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C6-Alkylthio-substituted purines have potential therapeutic applications such as in anticancer, antibacterial, and antiviral agents. For example, 4-nitrobenzylthioinosine (NBTI), which has been extensively studied in recent years, is a well-known inhibitor for the equilibrative nucleoside transport protein ENT1 (Figure 1).[1] Other C6-benzylthio-substituted purine nucleosides, which were similar to NBTI, have been reported to act as potential ATP-competitive kinase inhibitors,[2] antimycobacterial agents,[3] anti-HBV (hepatitis B virus) reagents,[4] and so on.[5] The importance of these C6-alkylthio substituted purine nucleosides in medicinal chemistry has promoted continued methodological efforts.

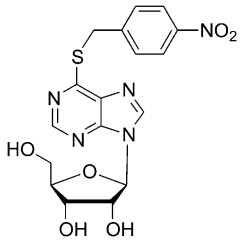

Figure 1. 4-Nitrobenzylthioinoisine (NBTI).

The preparation of C6-alkylthio-substituted purines mainly involves two different routes. One route is through an alkylation reaction from 6-mercaptopurine and alkylating agents such as alkyl halides,[6] and the other is through the S_NAr reaction of leaving groups from the heterocylic base by sulfurated nucleophiles such as thiols or RS^- anions.[7] As far as we know, thiourea, a most attractive starting material owing to its low cost and readily accessibility, is not used as a sulfur precursor for the preparation of C6-sulfur-substituted purines. Obviously, using thiourea as a sulfur source for synthesis of C6-sulfur-substituted purines would expand the scope of existing synthetic methodologies and overcompensate the disadvantages, which result from the relatively high cost of the starting material of 6-mercaptopurine, thiols, or RS^- anions.

In 2003, Peñéñory et al. have published "one-pot" two-step photoinduced reactions of aryl halides with the thiourea anion to afford aryl sulfur compounds.[8] Very recently, Sekar and Prasad have reported a one-pot synthesis of unsymmetrical diaryl thioethers from aryl halides and potassium ethyl xanthogenate catalyzed by $Cu(OAc)_2$.[9] Also, Firouzabadi et al. have developed a new protocol for the thioetherification of aryl halides using thiourea and alkyl bromides catalyzed by CuI in wet polyethylene glycol.[10] Encouraged by the unique properties of thiourea and based on the previous work of our studies on nucleoside analogues,[11] herein, we report a green and efficient protocol for the one-pot two-step synthesis of a series of the NBTI analogues from various 6-halopurine nucleosides and thiourea without any metal catalyst under microwave irradiated conditions. In this protocol, 2-(9*H*-purin-6-yl)-isothiourea hydrochloride was firstly formed by the formation of $C_{(aryl)}$–S bond without any catalyst. Next, a substitution reaction took place between 2-(9*H*-purin-6-yl)-isothiourea hydrochloride and an alkyl halide under microwave irradiation in 10 minutes to give C6-alkylthio-substituted purine nucleosides.

The first step was performed according the literature procedure[12]: thiourea (0.33 mmol) was added to a solution of purine **1a** (0.3 mmol) in ethanol (2 mL) and then the solution was refluxed at 95 °C for one hour to give the intermediate compound, 2-(9*H*-purin-6-yl)-isothiourea hydrochloride (**2a**). We also tried the reaction under microwave irradiation, however, unfortunately, the yield of **2a** was very low. Then, without the isolation of **2**, we studied the nucleophilic substitution reaction between **2a** and benzyl chloride (**3a**) promoted by microwave irradiation. The results are listed in Table 1.

As shown in Table 1, an excellent yield was obtained when two equivalents of benzyl chloride and two equivalents of K_2CO_3 were used (Table 1, entry 1). When the amount of benzyl chloride or K_2CO_3 was reduced, **4a** was afforded in lower yield (Table 1, entries 2–4). An exhilarating yield was obtained after the microwave power was increased to 400 W (Table 1, entry 5). When the reaction was performed at lower temperature or within a shorter time period, **4a** was obtained in lower yield (Table 1, entries 6–8). The reaction was also carried out in a convectional heating

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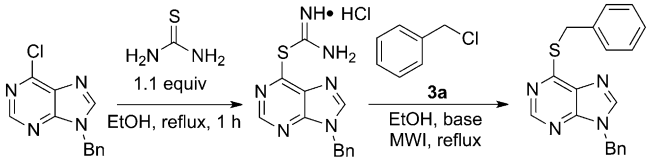
Table 1. Optimization of the Reaction Conditions[a]



Entry	Substrate **3a** [equiv]	K_2CO_3 [equiv]	Power [W]	*t* [min]	Yield [%][b]
1	2	2	300	10	95
2	2	1.5	300	10	88
3	2	–	300	10	0
4	1.5	2	300	10	85
5	**1.5**	**2**	**400**	**10**	**96**
6	1.2	2	400	10	72
7	1.5	2	400	8	83
8[c]	1.5	2	400	10	74
9[d]	1.5	2	–	60	84

[a] Reaction conditions: **1a** (0.3 mmol), EtOH (2 mL), MWI, reflux at 100 °C. [b] Yield of isolated product based on purine **1a**. [c] The reaction temperature was 50 °C. [d] The reaction was carried out in a convectional heating bath at 100 °C. MWI = microwave irradiation.

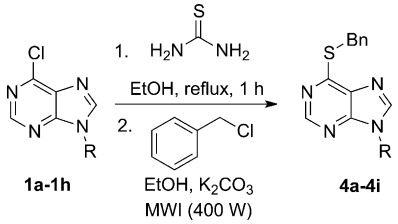
bath at 100 °C, and a similar result was obtained albeit with a longer reaction time (Table 1, entry 9).

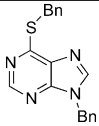
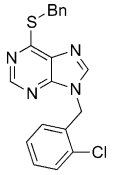
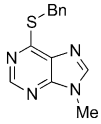
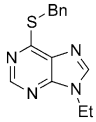
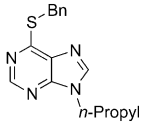
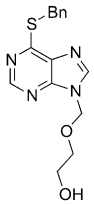
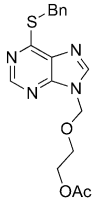
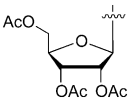
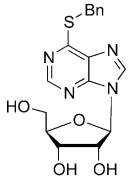
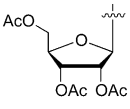
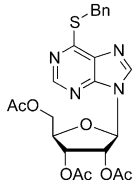
Under the optimized reaction conditions, the substrate scope was explored to various 6-chloropurine derivatives with different substituents at the N9 position. The results are summarized in Table 2. The substrates with various alkyl substituents at the N9 position gave high to excellent yields (Table 2, entries 1-5). To our delight, acyclic nucleoside acetic acid 2-(6-chloro-purin-9-ylmethoxy)-ethyl ester (**1f**) gave hydrolysis product (**4f**) in 80 % yield (Table 2, entry 6). To avoid the hydrolysis process, we simply replaced K_2CO_3 with Na_2CO_3, and the corresponding acetyl protected product (**4g**) was obtained in 83 % yield (Table 2, entry 7). 2',3',5'-Triacyl-6-chloropurine nucleoside (**1g**) gave the same results (Table 2, entries 8–9).

Using Na_2CO_3 as the base, various haloalkanes were subjected to the reactions with 6-chloropurine nucleoside (**1g**), and the results are shown in Table 3. It was found that all haloalkanes (Cl, Br, I) could smoothly produce the corresponding products in good to excellent yields. Therefore, this one-pot two-step synthetic method proved to be general for the synthesis of NBTI analogues.

This method also applies to other electron-deficient aryl halides, and the results are shown in Table 4. Several typical

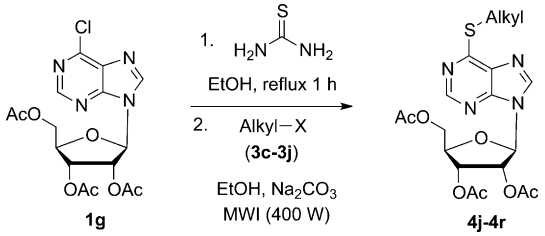
Table 2. Substrate Variation of 6-Chloropurines[a]



Entry	R	Product		Yield [%][b]
1	Bn (**1a**)		**4a**	96
2	*o*-Cl-Bn (**1b**)		**4b**	98
3	Me (**1c**)		**4c**	95
4	Et (**1d**)		**4d**	97
5	*n*-Propyl (**1e**)		**4e**	88
6			**4f**	80
7[c]			**4g**	83
8[d]			**4h**	84
9[c]			**4i**	93

[a] Reaction conditions of step one: purines **1** (0.3 mmol), thiourea (0.33 mmol), EtOH (2 mL), reflux at 100 °C for 1 h. Step two: benzyl chloride **3a** (0.45 mmol), K_2CO_3 (0.6 mmol), MWI 400 W, reflux at 100 °C for 10 min. [b] Yield of isolated product based on purines. [c] 2 equivalents of Na_2CO_3 were employed. [d] 3 equivalents of K_2CO_3 were employed.

Table 3. Substrate variation of haloalkanes[a]



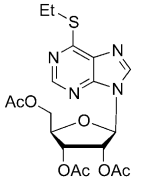
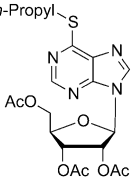
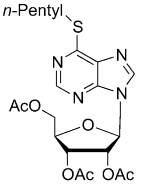
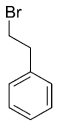
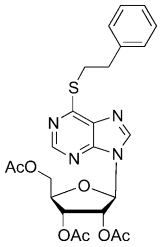
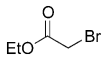
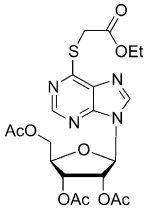
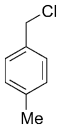
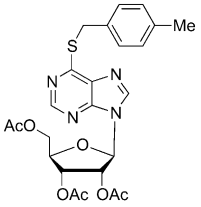
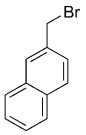
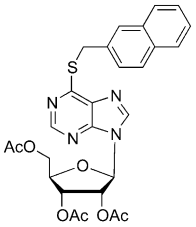
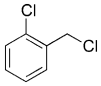
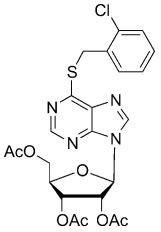
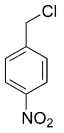
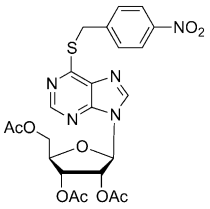
Entry	Alkyl-X	Product		Yield [%][b]
1	Et-I		**4j**	87
2	*n*-Propyl-I		**4k**	93
3	*n*-Pentyl-Br		**4l**	95
4			**4m**	99
5			**4n**	80

Table 3. (Continued)

Entry	Alkyl-X	Product		Yield [%][b]
6			**4o**	80
7			**4p**	85
8			**4q**	99
9			**4r**	81

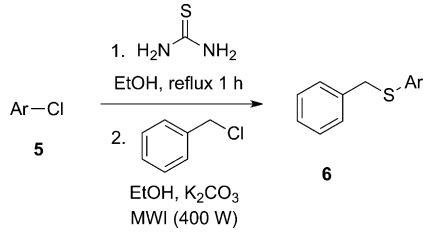
[a] Reaction conditions of step one: purines **1** (0.3 mmol), thiourea (0.33 mmol), EtOH (2 mL), reflux at 95 °C for 1 h. Step two: haloalkane **3** (0.45 mmol), Na_2CO_3 (0.6 mmol), MWI 400 W, reflux at 90 °C for 10 min. [b] Yield of isolated product based on purines.

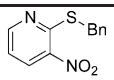
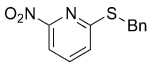
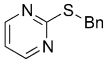
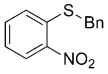
aryl and heteroaryl chlorides underwent the reactions smoothly with good yields (Table 4, entries 1–4). Iodobenzene and some electron-rich aromatics, such as *para*-methylphenyl bromide and *meta*-bromoanisole were not successful in the reaction, and the corresponding products were not obtained.

Finally, we used this protocol to synthesize 4-nitrobenzylthioinosine (NBTI, **7**), a well-known inhibitor for the nucleoside transport protein ENT1. To our delight, NBTI was efficiently achieved in an isolated yield of 87% by using this one-pot two-step method (Scheme 1).

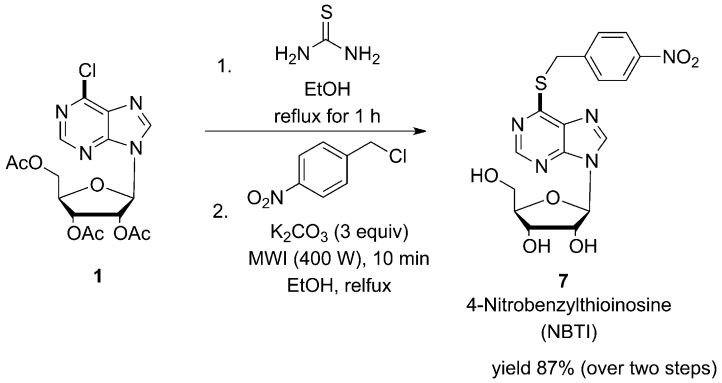
In conclusion, we have developed a rapid and operationally simple method for the preparation of various 6-thio-substituted purine nucleosides, which are important candidates for biologically active compounds. Thiourea, an easily available and cheap starting material, was first used as a sulfur precursor for synthesis of 6-thio-substituted purine nucleosides. In this protocol, isothiourea hydrochloride was firstly formed by formation of the $C_{(aryl)}$–S bond without any catalyst, and subsequently, a substitution reaction took place between an alkyl halide and the intermediate product under microwave irradiation in 10 minutes to give the 6-thio-substituted purine nucleosides. One of the important applications to showcase such a mild and efficient protocol is the synthesis of the biologically important compound, NBTI. This methodology offers an important complement to the reported methods for synthesis of 6-thio-substituted purine nucleosides from thiourea without any metal catalyst.

Table 4. Substrate variation of heteroaromatics[a]



Entry	Product		Yield [%][b]
1		**6a**	80
2		**6b**	85
3		**6c**	75
4		**6d**	88

[a] Reaction conditions of step one: aryl chloride **5** (0.3 mmol), thiourea (0.33 mmol), EtOH (2 mL), reflux at 95 °C for 1 h. Step two: haloalkane **3** (0.45 mmol), K_2CO_3 (0.6 mmol), MWI 400 W, reflux at 90 °C for 10 min. [b] Yield of isolated product based on aryl halides.



Scheme 1. Convenient Synthesis of 4-Nitrobenzylthioinosine (NBTI).

Experimental Section

Typical Experimental Procedure

Purine **1** (0.5 mmol) was placed in a 10 mL glass vial equipped with a small magnetic stirring bar. Then ethanol (2 mL) and thiourea (1.5 equiv) were added and the solution was refluxed for 1 h. *S*-(purin-6-yl)isothiourea hydrochloride **2** was formed as a precipitate. Then the haloalkane (1.5 equiv) and K_2CO_3 (2 equiv) were added (for **4a**–**4f** and **6a**–**6d**). For **4h** and **7** K_2CO_3 (3 equiv) was added. For **4g** and **4i**–**4r** Na_2CO_3 (2 equiv) was added. Then the mixture was put into the cavity of the microwave and irradiated at 400 W at 90 °C for 10 min. After evaporation of the solvent, the crude product was purified by column chromatography over silica gel using EtOAc/Petroleum Ether (v/v = 1:4) as the eluent to give desired products **4a**–**4e**, **4g**, and **6a**–**6d**, EtOAc/Petroleum Ether (v/v = 1:2) was used as the eluent to give desired products **4f** and **4i**–**4r**, and EtOAc/Methanol (v/v = 50:1) was used as the eluent to give desired products **4h** and **7**.

*Compound **4a***

White powder, m.p. 101–103 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.79 (s, 1H), 8.04 (s, 1H), 7.48–7.46 (m, 2H), 7.34–7.21 (m, 8H), 5.41 (s, 2H), 4.68 ppm (s, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 160.5, 151.9, 148.5, 142.5, 137.2, 134.9, 130.4, 129.1, 129.0, 128.5, 128.4, 127.7, 127.2, 47.3, 32.8 ppm.

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