



Facile and efficient access to 2,6,9-tri-substituted purines through sequential N9, N2 Mitsunobu reactions

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ABSTRACT

A facile, efficient and mild synthesis of 2,6,9-tri-substituted purines is presented, starting from commercially available 2-amino-6-chloropurine, which employs sequential N9 then N2 Mitsunobu reactions as key steps. Importantly, our synthetic approach to N2-functionalization of the purine nucleus obviates the harsh conditions required by the traditional nucleophilic aromatic substitution of a 2-halo group with primary amines. Benzylic, allylic, propargylic and aliphatic alcohols all coupled in very good to excellent yields in both Mitsunobu reactions. Significantly, excellent chemoselectivity and N9-regioselectivity were observed for the first coupling, and reactions were complete within 15 min at room temperature. Our novel methodology may be readily adapted to furnish N^9 -mono- or N^2,N^9 -di-functionalized guanine analogues, and the utility of our protocol is further demonstrated by the efficient synthesis of the CDK inhibitor bohemine.

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Protein kinases, which play critical roles in regulating the cell cycle, utilize ATP as a co-substrate, and thus considerable research into the design of kinase inhibitors has focused on the development of purine-based ATP antagonists.¹ Notably, 2,6,9-tri-substituted purines, including olomoucine, bohemine and (*R*)-roscovitine (Fig. 1), have proven potent and selective inhibitors of cyclin-dependent kinases (CDKs).² In fact, Cyclacel Pharmaceuticals has recently announced that (*R*)-roscovitine has progressed to Phase IIb clinical trials against non-small cell lung cancer, and to Phase II against nasopharyngeal cancer.³

The synthesis of 2,6,9-tri-substituted purines typically begins with 2,6-dihalopurines. Whilst displacement of the 6-halo (usually chloro) group with amine nucleophiles is relatively facile, nucleophilic aromatic substitution of the 2-halo group often demands a large excess of the amine, high temperatures and/or extended reaction times.⁴ Excepting recent research in microwave-assisted synthesis,⁵ milder alternatives to the synthesis of 2,6,9-tri-substituted purines would be of significant benefit. As part of our research on peptide nucleic acids (PNAs), we have developed a synthetic route for the preparation of novel N^2,N^9 -di-functionalized guanines starting from the popular guanine precursor 2-amino-6-chloropurine (**1**), which we have found may be readily adapted to the preparation of other purines. In this Letter, we wish to report a novel methodology for the facile and efficient synthesis of 2,6,9-tri-substituted purines, including N^9 -mono and N^2,N^9 -di-functionalized guanines, which exploits mild, sequential Mitsunobu reactions as key steps.

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Direct N9-functionalization of **1** under Mitsunobu conditions⁶ has been reported previously, and, although good N9-regioselectivity was observed, products were generally obtained in poor to moderate yields,^{7a-c} with higher yields being obtained when several equivalents of reagents were employed.^{7d} The poor solubility of **1** in THF, the preferred solvent for Mitsunobu reactions, and the competing nucleophilicity of the exocyclic N2 amino group are likely primary reasons for the limited yields observed.

We reasoned that protection of the amine of **1** with a hydrophobic group such as *tert*-butoxycarbonyl (Boc) should enhance its solubility in organic solvents. In addition, the resultant NHBoc carbamate should function as an activated amine suitable for participation in the Mitsunobu reaction, wherein the inherent bulk of the Boc group should, at the same time, exert chemoselective control, allowing the Mitsunobu reaction to proceed first at the endocyclic N9 position and then, in a subsequent step, at the exocyclic N2 position. To this end, treatment of **1** with 1 equiv of Boc₂O in the presence of catalytic DMAP in DMSO led to N^9 -Boc-2-amino-6-chloropurine (**2**) quantitatively within 30 min (Scheme 1). Although here not significant, the N9 versus N7 regiochemistry was assumed, based on the reported N9 regiochemistry of the tris-Boc protected analogue of **2**.⁸ Generation of the anilide anion of **2** with an excess of NaH in THF invoked the desired shift of the Boc group from N9 to N2, furnishing N^2 -Boc-2-amino-6-chloropurine (**3**) in near-quantitative yield, and thereby providing a higher yielding and more economical route to the previously reported compound **3**.⁸

Purine **3** exhibited much improved solubility in organic solvents, especially in THF, and, employing *n*-butanol as a representative alcohol, the desired Mitsunobu reaction proceeded in both excellent yield, chemoselectivity and N9-regioselectivity⁹ (**4a**: R¹ = *n*-Bu, 88%), with the diisopropylazodicarboxylate

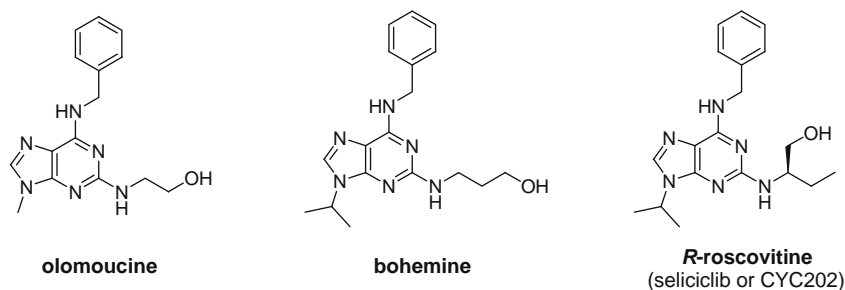


Figure 1. 2,6,9-Tri-substituted purine CDK inhibitors.

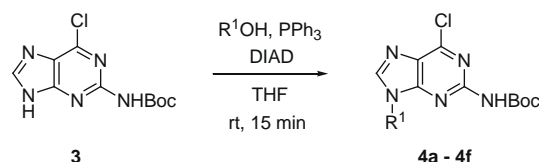
(DIAD)/triphenylphosphine (PPh₃) redox combination (Scheme 2). The excellent N9-regioselectivity is consistent with the findings of others working on related purines.¹⁰

Reports on the Mitsunobu-mediated alkylation of the purine N2 amino group are scarce. Of those reports, activation was achieved by converting the amino group to its acetamide^{11,12} or to its more reactive (more acidic) trifluoroacetamide.¹³ Yields were poor to moderate with aliphatic alcohols;^{11,13} highest yields were limited to allylic and benzylic alcohols.¹² The use of the Boc group to protect/activate the aromatic amino group of functionalized heterocycles towards the Mitsunobu reaction has also been reported in moderate yield,¹⁴ giving us confidence that the likewise alkylation of the NHBoc unit in purine **4** may be successful. Indeed, **4** was smoothly alkylated with *n*-butanol under modified Mitsunobu conditions (2.5 equiv of each of *n*-butanol, PPh₃ and DIAD at 35 °C for 30 min) to give **5aa** (R¹ = R² = *n*-Bu) in excellent yield (93%). Nucleophilic aromatic substitution of **5aa** with benzylamine, followed by deprotection of the Boc group with TFA furnished the 2,6,9-tri-substituted purine **6aa** (R¹ = R² = *n*-Bu, R³ = Bn) in a noteworthy overall yield of 72% (six steps).

Given the success of the Mitsunobu-mediated, N9-functionalization of purine **3** with *n*-butanol, we were interested in examining the scope of this reaction. The results of our investigations are shown in Table 1. Primary and secondary aliphatic, allylic, propargylic and benzylic alcohols all gave very good to excellent yields of

Table 1

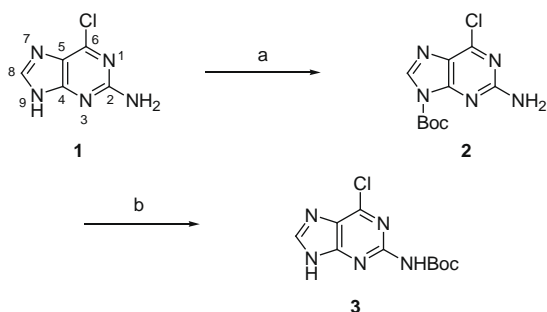
Reaction substrate scope in the N9 Mitsunobu coupling of purine **3** with R¹OH alcohols^a



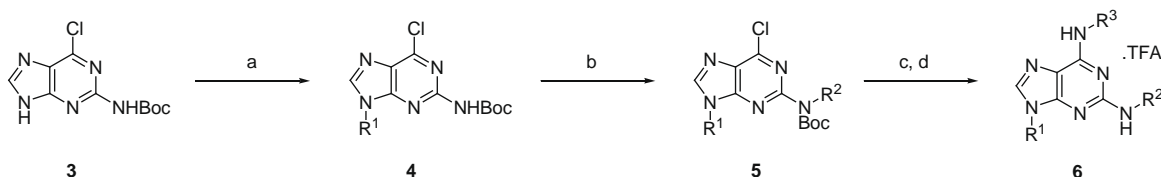
Entry	R ¹ OH	Product	Yield ^b (%)
1		4a	88
2		4b	89
3		4c	90
4		4d	86
5		4e	85
6		4f	85

^a Reaction conditions: purine **3a** (1 equiv), R¹OH (1.1 equiv) and PPh₃ (1.1 equiv) were dissolved in anhydrous THF (0.07 M) at rt. After 2 min, DIAD (1.1 equiv) was added dropwise.

^b Isolated yield after silica gel flash column chromatography.



Scheme 1. Reagents and conditions: (a) Boc₂O, cat. DMAP, DMSO, 0 °C, 30 min, 99%; (b) NaH, THF, rt, 2 h, 96%.



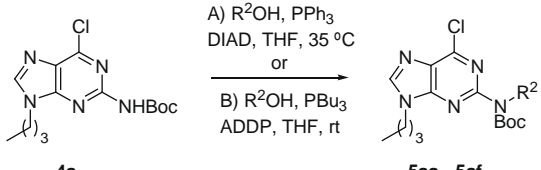
Scheme 2. Reagents and conditions: (a) (1) R¹OH, PPh₃, THF, rt, 2 min; (2) DIAD, rt, 15 min; (b) (1) R²OH, PPh₃, THF, rt, 2 min; (2) DIAD, 35 °C, 30 min; or (1) R²OH, PBu₃, THF, rt, 2 min; (2) ADDP, rt, 4–8 h; (c) R³NH₂, DIPEA, 75 °C, 6 h; (d) TFA/CH₂Cl₂, 1:1, rt, 1 h.

are significantly milder than those reported in a recent, N9 Mitsunobu methodology paper on a related purine derivative, likely due in part to the enhanced solubility of our purine substrate.¹⁰

As shown in Table 2, we then examined the ability of the N2 NHBoc group in **4a** to undergo the Mitsunobu reaction with the same set of alcohols featured in Table 1. In this case, two different reaction conditions were required, dependent on the nature of the alcohol, in order for all purine **4a** to be consumed. After some experimentation, it was found that the less reactive primary and secondary aliphatic alcohols *n*-butanol, 3-(*tert*-butyldimethylsilyloxy)-propanol and *iso*-propanol (2.5 equiv) all effected smooth conversion of **4a** (1 equiv) to the products **5aa**, **5ab** and **5ac**, respectively, with the DIAD/PPh₃ redox system (2.5 equiv of each) at 35 °C. Reactions proceeded in excellent yields and were complete within 30 min (Table 2, entries 1–3). In contrast, treatment of purine **4a** with the more reactive allylic, propargylic and benzylic alcohols using the previously described protocol led to around only 70% of the starting material being consumed. In addition to the steric hindrance about the NHBoc group, which was effectively countered, in the case of the aliphatic alcohols, by employing an excess of the Mitsunobu reagents to compensate for their consumption in side reactions, we also considered that the pK_a of the NHBoc group might be on the cusp of the ‘allowed pK_a’ for the Mitsunobu reaction. Thus, we investigated the alternative 1,1'-azodicarbonyldipiperidine (ADDP)/tri-*n*-butylphosphine (TBP) combination, which has been shown to successfully couple primary alcohols to less acidic HA nucleophiles (ADDP forms a more basic betaine than DIAD).¹⁶ Gratifyingly, after optimization, we found that allylic, propargylic and benzylic alcohols all coupled smoothly with purine **4a** in the presence of 2.5 equiv of the alcohol, ADDP and PBu₃, proceeding to completion over 4–8 h at room temperature and in very good yields (Table 2, entries 4–6).

Nucleophilic aromatic substitution of 6-chloropurines with primary amines is well documented.¹⁷ Indeed, reaction of compounds

Table 2
Reaction substrate scope in the N2 Mitsunobu coupling of purine **4a** with R²OH alcohols^a



Entry	R ² OH	Method	Time	Product	Yield ^b (%)
1		A	30 min	5aa	93
2		A	30 min	5ab ^c	93
3		A	30 min	5ac	92
4		B	8 h	5ad	86
5		B	8 h	5ae	88
6		B	4 h	5af	87

^a Reaction conditions: method (A) purine **4a** (1 equiv), R²OH (2.5 equiv) and PPh₃ (2.5 equiv) were dissolved in anhydrous THF (0.07 M) at rt. After 2 min, DIAD (2.5 equiv) was added dropwise, then reaction was heated at 35 °C for 30 min. Method (B) purine **4a** (1 equiv), R²OH (2.5 equiv) and PBu₃ (2.5 equiv) were dissolved in anhydrous THF (0.07 M). After 2 min, ADDP (2.5 equiv) was added in one portion. Reaction was stirred at rt for time indicated.

^b Isolated yield after silica gel flash column chromatography.

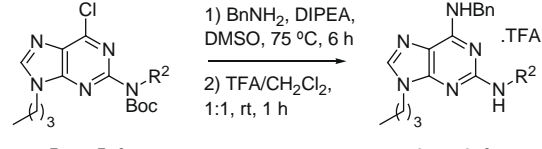
^c Product **5ab** contaminated with ca. 10% DIAD-H₂ by-product.

5aa–af with benzylamine as a representative primary amine proceeded in excellent yields, and subsequent Boc deprotections with 50% TFA in CH₂Cl₂ were quantitative (Table 3, yields are for two steps), furnishing the 2,6,9-tri-substituted purines **6aa–af** as their TFA salts.¹⁸ 6-Chloropurine derivatives such as **5** are versatile intermediates; the chlorine atom can be readily displaced by other nucleophiles, including alcohols,^{17,19} thiols^{17,20} and water.^{17,7b} For example, quantitative hydrolytic dechlorination^{7b} of **4a** or **5aa** with TFA/H₂O afforded the N⁹-functionalized guanine **7a** or the N²,N⁹-di-functionalized guanine **7aa**, respectively (Scheme 3), demonstrating how our methodology may be readily adapted to the synthesis of novel guanine analogues.

Finally, we wish to report the utility of our methodology in the synthesis of the 2,6,9-tri-substituted purine CDK inhibitor bohemine (**8cb**), which was thus furnished in excellent overall yield (64%) from commercially available purine **1** (Scheme 4).

In conclusion, we have developed a novel methodology for the facile and efficient synthesis of 2,6,9-tri-substituted purines, including N⁹-mono- and N²,N⁹-di-functionalized guanines, which exploits mild, sequential Mitsunobu reactions as key steps in the syntheses. Benzylic, allylic, propargylic and aliphatic alcohols all

Table 3
C6 Nucleophilic aromatic substitution followed by Boc-deprotection of purines **5aa–af** to furnish 2,6,9-tri-substituted purines **6aa–af**^a

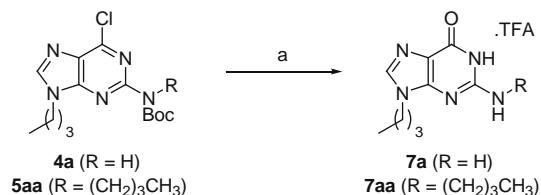


Entry	Substrate	R ²	Product	Yield ^b (%)
1	5aa		6aa	92
2	5ab		6ab ^c	85
3	5ac		6ac	93
4	5ad		6ad	90
5	5ae		6ae	91
6	5af		6af	93

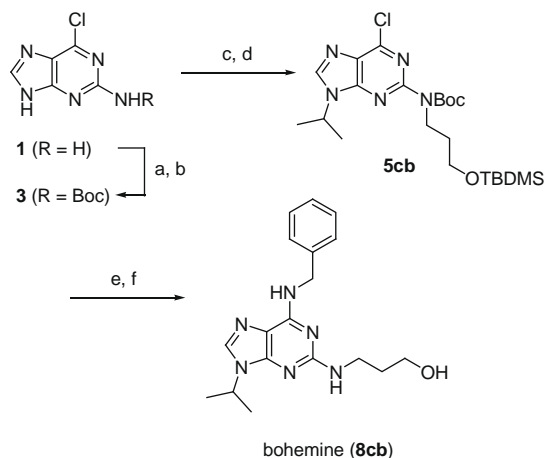
^a Reaction conditions: (1) a solution of purine **5aa** (1 equiv), DIPEA (3 equiv) and BnNH₂ (1.5 equiv) in anhydrous DMSO (0.15 M) was heated at 75 °C for 6 h; (2) Boc-protected analogue of purine **6aa** was deprotected in a 1:1 mixture of TFA/CH₂Cl₂ (0.05 M) at rt for 1 h.

^b Isolated yield (over two steps) of TFA salt.

^c Partial cleavage of the TBDMS group was also observed after 1 h in TFA/CH₂Cl₂; complete removal was accomplished by extending the reaction time to 2 h. Product **6ab** was furnished as the free base after work-up and purification by silica gel flash column chromatography.



Scheme 3. Reagents and conditions: (a) TFA/H₂O, 3:1, rt, 48 h, 100%.



Scheme 4. Reagents and conditions: (a) Boc_2O , cat. DMAP, DMSO, 0 °C, 30 min, 99%; (b) NaH, THF, rt, 2 h, 96%; (c) (1) *i*-PrOH, PPh_3 , THF, rt, 2 min; (2) DIAD, rt, 15 min, 90%; (d) (1) TBDMSCl , $\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$, PPh_3 , THF, rt, 2 min; (2) DIAD, 35 °C, 30 min, 93%; (e) BnNH_2 , DIPEA, DMSO, 75 °C, 6 h, 91%; (f) TFA/ CH_2Cl_2 , 1:1, rt, 2 h, 89%.

coupled in very good to excellent yields in both reactions, the first of which was swift (reactions were complete within 15 min), chemoselective and N9-regioselective. Significantly, our synthetic approach to N2-functionalization of the purine nucleus obviates the harsh conditions required by the traditional nucleophilic aromatic substitution of a 2-halo group with primary amines.

Acknowledgments

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Supplementary data

Supplementary data (synthetic procedures and characterization data (^1H NMR, ^{13}C NMR and MS) for purines **2**, **3**, **4a**, **5aa**, **5af** and

8cb) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.04.137.

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