Tetrahedron Letters 50 (2009) 4258–4261

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

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

Steven Fletcher *, Vijay M. Shahani, Patrick T. Gunning *

*Department of Chemistry, University of Toronto, Mississauga, ON, L5L 1C6, Canada*

ARTICLE INFO

*Article history:*
Received 8 April 2009
Revised 29 April 2009
Accepted 30 April 2009
Available online 6 May 2009

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.



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.

Direct N9-functionalization of **1** under Mitsunobu conditions[6] 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 $Boc_2O$ 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-regioselectivity[9] (**4a**: $R^1$ = *n*-Bu, 88%), with the di*iso*propylazodicarboxylate

* Corresponding authors. Tel.: +1 905 569 4588; fax: +1 905 569 4929 (P.T.G.).
*E-mail addresses:* steven.fletcher@utoronto.ca (S. Fletcher), patrick.gunning@utoronto.ca (P.T. Gunning).

0040-4039/$ - see front matter Crown Copyright © 2009 Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2009.04.137

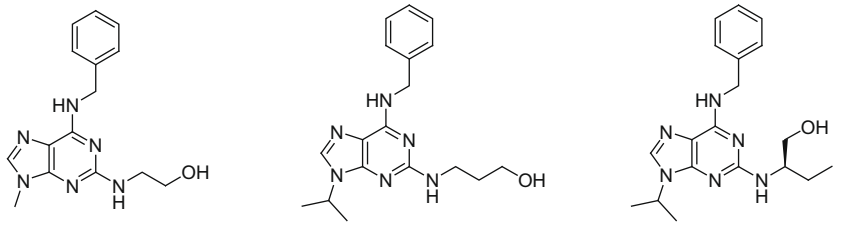
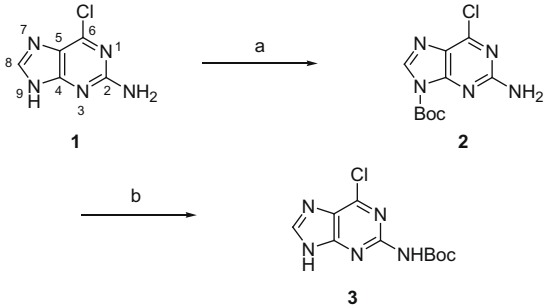



**Figure 1.** 2,6,9-Tri-substituted purine CDK inhibitors.

(DIAD)/triphenylphosphine ($PPh_3$) redox combination (Scheme 2). The excellent N9-regioselectivity is consistent with the findings of others working on related purines.[10]

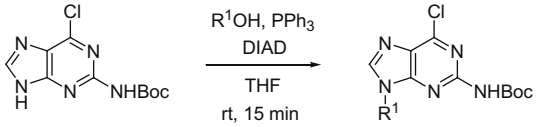
Reports on the Mitsunobu-mediated alkylation of the purine N2 amino group are scarce. Of those reports, activation was achieved by converting the amino group to its acetamide[11,12] or to its more reactive (more acidic) trifluoroacetamide.[13] Yields were poor to moderate with aliphatic alcohols;[11,13] 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, $PPh_3$ and DIAD at 35 °C for 30 min) to give **5aa** ($R^1 = R^2 = n$-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** ($R^1 = R^2 = n$-Bu, $R^3$ = 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

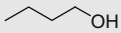
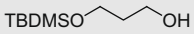
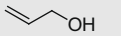
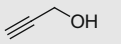
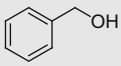



**Scheme 1.** Reagents and conditions: (a) $Boc_2O$, cat. DMAP, DMSO, 0 °C, 30 min, 99%; (b) NaH, THF, rt, 2 h, 96%.

**Table 1**
Reaction substrate scope in the N9 Mitsunobu coupling of purine **3** with $R^1OH$ alcohols[a]

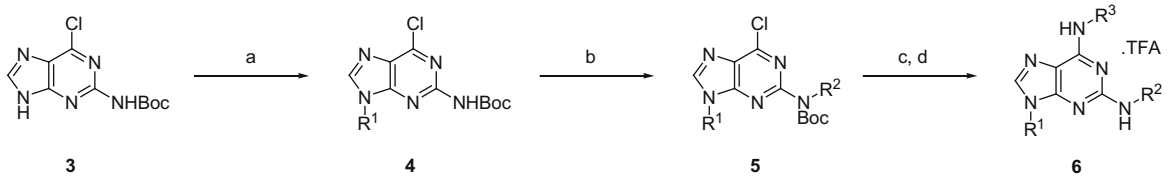



**3** **4a - 4f**

| Entry | $R^1OH$ | 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), $R^1OH$ (1.1 equiv) and $PPh_3$ (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.

N9 alkylated products, with only trace to minor amounts (<5–10%) of the more polar (TLC, silica gel) N7 alkylated products observed.[15] N9-Regioselectivity was confirmed by comparing the $^1H$ and $^{13}C$ NMR spectra of the two regioisomers, as described in the literature.[9] Importantly, reaction conditions were especially mild, requiring just 1.1 equiv of each of the alcohol, $PPh_3$ and DIAD, and proceeding to completion within 15 min at room temperature, suggesting this Mitsunobu reaction should also be compatible with more complex purine substrates. Moreover, it is noteworthy that, whilst yields are approximately the same, our reaction conditions




**Scheme 2.** Reagents and conditions: (a) (1) $R^1OH$, $PPh_3$, THF, rt, 2 min; (2) DIAD, rt, 15 min; (b) (1) $R^2OH$, $PPh_3$, THF, rt, 2 min; (2) DIAD, 35 °C, 30 min; or (1) $R^2OH$, $PBu_3$, THF, rt, 2 min; (2) ADDP, rt, 4–8 h; (c) $R^3NH_2$, DIPEA, 75 °C, 6 h; (d) TFA/$CH_2Cl_2$, 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]

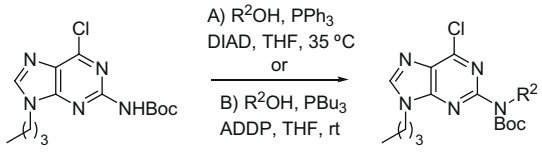
As shown in Table 2, we then examined the ability of the N2 NHBoc group in **4a** to undergo the Mitsunobu reaction with the same set of alcohols featured in Table 1. In this case, two different reaction conditions were required, dependent on the nature of the alcohol, in order for all purine **4a** 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/$PPh_3$ 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, progargylic 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 p$K_a$ of the NHBoc group might be on the cusp of the 'allowed p$K_a$' 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 $PBu_3$, 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 **5aa**–**af** with benzylamine as a representative primary amine proceeded in excellent yields, and subsequent Boc deprotections with 50% TFA in $CH_2Cl_2$ 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/$H_2O$ afforded the $N^9$-functionalized guanine **7a** or the $N^2$,$N^9$-di-functionalized guanine **7aa**, respectively (Scheme 3), demonstrating how our methodology may be readily adapted to the synthesis of novel guanine analogues.

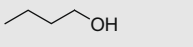
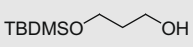
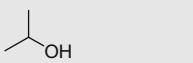
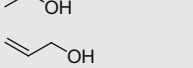
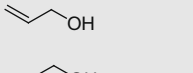
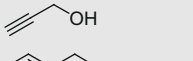
Finally, we wish to report the utility of our methodology in the synthesis of the 2,6,9-tri-substituted purine CDK inhibitor bohemine (**8cb**), which was thus furnished in excellent overall yield (64%) from commercially available purine **1** (Scheme 4).

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

**Table 2**
Reaction substrate scope in the N2 Mitsunobu coupling of purine **4a** with $R^2OH$ alcohols[a]

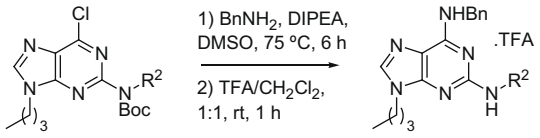



**4a** **5aa - 5af**

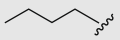
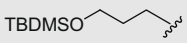
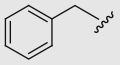
| Entry | $R^2OH$ | Method | Time | Product | Yield[b] (%) |
|---|---|---|---|---|---|
| 1 |  | A | 30 min | **5aa** | 93 |
| 2 |  | A | 30 min | **5ab**[c] | 93 |
| 3 |  | A | 30 min | **5ac** | 92 |
| 4 |  | B | 8 h | **5ad** | 86 |
| 5 |  | B | 8 h | **5ae** | 88 |
| 6 |  | B | 4 h | **5af** | 87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

[a] Reaction conditions: method (A) purine **4a** (1 equiv), $R^2OH$ (2.5 equiv) and $PPh_3$ (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), $R^2OH$ (2.5 equiv) and $PBu_3$ (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-$H_2$ by-product.

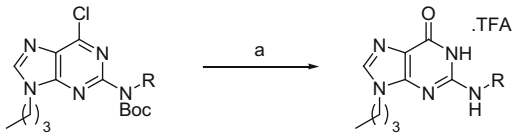
**Table 3**
C6 Nucleophilic aromatic substitution followed by Boc-deprotection of purines **5aa**–**af** to furnish 2,6,9-tri-substituted purines **6aa**–**af**[a]




**5aa - 5af** **6aa - 6af**

| 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 |

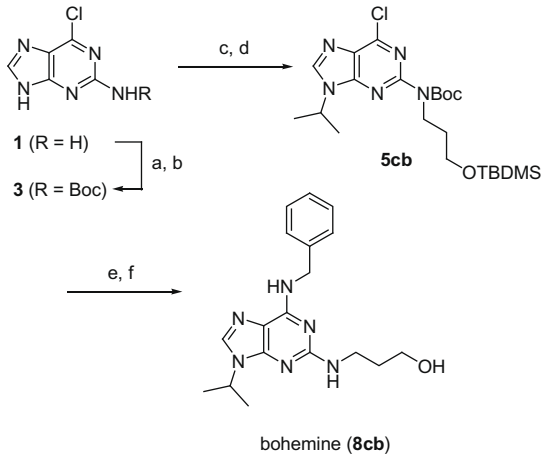
[a] Reaction conditions: (1) a solution of purine **5aa** (1 equiv), DIPEA (3 equiv) and $BnNH_2$ (1.5 equiv) in anhydrous DMSO (0.15 M) was heated at 75 °C for 6 h; (2) Boc-protected analogue of purine **6aa** was deprotected in a 1:1 mixture of TFA/$CH_2Cl_2$ (0.05 M) at rt for 1 h.
[b] Isolated yield (over two steps) of TFA salt.
[c] Partial cleavage of the TBDMS group was also observed after 1 h in TFA/$CH_2Cl_2$; 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.




**4a** (R = H)
**5aa** (R = $(CH_2)_3CH_3$)

**7a** (R = H)
**7aa** (R = $(CH_2)_3CH_3$)

**Scheme 3.** Reagents and conditions: (a) TFA/$H_2O$, 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) $TBDMSOCH_2CH_2CH_2OH$, $PPh_3$, 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

The authors gratefully acknowledge financial support for this work from the Canadian Foundation of Innovation and the University of Toronto (Connaught Foundation).

## 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.

## References and notes

1. Legraverend, M.; Grierson, D. S. *Bioorg. Med. Chem.* **2006**, *14*, 3987–4006. and references 132–152 therein.
2. Haesslein, J. L.; Jullian, N. *Curr. Top. Med. Chem.* **2002**, *2*, 1037–1050.
3. www.cyclacel.com/cyc/investors/news/pressreleases/2006/2006-06-29 www.cyclacel.com/cyc/investors/news/pressreleases/2009/2009-03-05.
4. Bork, J. T.; Lee, J. W.; Chang, Y.-T. *QSAR Comb. Sci.* **2004**, *23*, 245–260.
5. (a) Austin, R. E.; Okonya, J. F.; Bond, D. R. S.; Al-Obeidi, F. *Tetrahedron Lett.* **2002**, *43*, 6171–6196; (b) Huang, H.; Liu, H.; Chen, K.; Jiang, H. *J. Comb. Chem.* **2007**, *9*, 197–199.
6. Mitsunobu, O. *Synthesis* **1981**, *1*, 1–28.
7. (a) Choo, H.; Beadle, J. R.; Chong, Y.; Trahan, J.; Hostetler, K. Y. *Bioorg. Med. Chem.* **2007**, *15*, 1771–1779; (b) Tang, Y.; Muthyala, R.; Vince, R. *Bioorg. Med. Chem.* **2006**, *14*, 5866–5875; (c) Hikishima, S.; Isobe, M.; Koyanagi, S.; Soeda, S.; Shimeno, H.; Shibuya, S.; Yokomatsu, T. *Bioorg. Med. Chem.* **2006**, *14*, 1660–1670; (d) Kim, H. S.; Ohno, M.; Xu, B.; Kim, H. O.; Choi, Y.; Ji, X. D.; Maddileti, S.; Marquez, V. E.; Harden, T. K.; Jacobson, K. A. *J. Med. Chem.* **2003**, *46*, 4974–4987.
8. Dey, S.; Garner, P. *J. Org. Chem.* **2000**, *65*, 7697–7699.
9. N9-Regioselectivity was confirmed by comparing the $^1H$ and $^{13}C$ NMR spectra (in DMSO-$d_6$) of the N9 and N7 isomers, as according to Kjellberg, J.; Johansson, N. G. *Tetrahedron* **1986**, *42*, 6541–6544.
10. Lu, W.; Sengupta, S.; Petersen, J. L.; Akhmedov, N. G.; Shi, X. *J. Org. Chem.* **2007**, *72*, 5012–5015.
11. Maruyama, T.; Yorikane, A.; Kozai, S. *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 935–936.
12. Véliz, E. A.; Beal, E. A. *Tetrahedron Lett.* **2006**, *47*, 3153–3156.
13. Chang, Y.-T.; Gray, N. S.; Rsania, G. R.; Sutherlin, D. P.; Kwon, S.; Norman, T. C.; Sarohia, R.; Leost, M.; Meijer, L.; Schultz, P. G. *Chem. Biol.* **1999**, *6*, 361–375.
14. Perron-Sierra, F.; Dizier, D. S.; Bertrand, M.; Genton, A.; Tucker, G. C.; Casara, P. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3291–3296.
15. For example, N9-**4f**: $R_f = 0.46$ (Hex/EtOAc, 1:9); N7-**4f**: $R_f = 0.17$ (Hex/EtOAc, 1:9). Our findings that N7-alkylated purines are more polar than their regioisomeric N9 counterparts is consistent with reports in the literature, such as: Geen, G. R.; Grinter, T. J.; Kincey, P. M.; Jarvest, R. L. *Tetrahedron* **1990**, *46*, 6903–6914.
16. Tsunoda, T.; Yamamiya, Y.; Itô, S. *Tetrahedron Lett.* **1993**, *34*, 1639–1642.
17. For a review, see: Legraverend, M. *Tetrahedron* **2008**, *64*, 8585–8603.
18. Purines **6aa**, **6ac**–**af** as their TFA salts were of >95% purity, as judged by $^1H$ NMR. The purine·TFA salt can be neutralized and extracted into an organic solvent ($CH_2Cl_2$ or EtOAc) by partitioning against a saturated aqueous solution of $NaHCO_3$.
19. For example: Lembicz, N. K.; Grant, S.; Clegg, W.; Griffin, R. J.; Heath, S. L.; Golding, B. T. *J. Chem. Soc., Perkin Trans. 1* **1997**, 185–186.
20. For example: Sekiya, K.; Takashima, H.; Ueda, N.; Kamiya, N.; Yuasa, S.; Fujimura, Y.; Ubasawa, M. *J. Med. Chem.* **2002**, *45*, 3138–3142.