Tetrahedron Letters 51 (2010) 2948–2950

Contents lists available at ScienceDirect

Tetrahedron Letters

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

Regioselective alkylation of the exocyclic nitrogen of adenine and adenosine by the Mitsunobu reaction

Steven Fletcher *

Department of Pharmaceutical Sciences, University of Maryland School of Pharmacy, Baltimore, MD 21201, USA

ARTICLE INFO

Article history:
Received 2 March 2010
Revised 18 March 2010
Accepted 23 March 2010
Available online 27 March 2010

ABSTRACT

A novel synthetic route to N^6-substitution of adenine is presented, employing the Mitsunobu reaction as the key step. A range of primary and secondary alcohols all coupled in very good to excellent yields within 30 min at 45 °C, offering a milder alternative to the traditional nucleophilic aromatic substitution of 6-chloropurine. The utility of this protocol is further demonstrated by its application to the syntheses of N^6,N^9-di-substituted adenines, including the potent and selective A_1 adenosine receptor agonist N^6-cyclopentyladenosine.

Adenosine is an endogenous hormone that elicits both physiological and pathophysiological functions through binding to adenosine receptors (ARs). There are four known subtypes of adenosine receptor: A_1 and A_3, both of which inhibit the enzyme adenylate cyclase, leading to a reduction in cellular cAMP levels, and A_{2A} and A_{2B}, both of which stimulate adenylate cyclase, thereby raising the levels of cAMP.[1] The A_1 receptor plays a role in the regulation of heart rate; adenosine is an A_1 agonist, and is used in the treatment of tachycardia. The ubiquitous nature of adenosine receptors and the lack of receptor subtype selectivity have prompted the development of compounds that are selective for specific receptor subtypes. For example, it is known that functionalization of the exocyclic (N6) amino group of adenosine with heterocycles and cycloalkyls, as in N^6-cyclopentyladenosine (**1**, Fig. 1), enhances the specificity for the A_1 receptor.[2] Conversely, replacement of the ribose moiety in **1** with hydrophobic groups, including methyl, ethyl and cyclopentyl, to afford N^6,N^9-disubstituted adenines, such as **2** (Fig. 1), leads to the generation of potent and selective A_1 antagonists.[3] Meanwhile, N^6-substituted adenines, such as **3** (Fig. 1), are known synthetic cytokinins[4] as well as potent antimicrobial agents.[5]

N^6-Alkylation of adenines and adenosines is typically accomplished by nucleophilic aromatic substitution reactions with primary amines on the corresponding 6-chloropurines.[6] However, 6-chloropurine and 6-chloropurine riboside are considerably more expensive chemicals than their adenine counterparts. In addition, the conditions required for such reactions often involve an excess of the amine, high temperatures and reaction times, in some cases, upwards of 24 h. These conditions might be incompatible with certain substrates. An alternative synthetic approach might be the direct alkylation of the exocyclic amino group but methylation of adenosine leads to reaction at the 1- and 7-positions, with no alkylation of the N6 position.[7] Moreover, the attempted ethylation of adenosine also leads to a mixture of products.[7] A more common alkylation approach is described by Robins and Trip who effected the indirect N^6-alkylation of 2′-deoxyadenosine by N^1-alkylation followed by the Dimroth rearrangement to the desired N^6-substituted product.[8] More recently, Pedersen and co-workers have demonstrated a two-step, direct N^6-alkylation of 2′-deoxyadenosine using benzotriazole as a synthetic auxiliary, although overall yields are typically moderate.[9] Alkylation of the N6 amino group has also been accomplished by the reaction of N^6-benzoyladenosine derivatives with the highly reactive electrophile benzyloxymethyl chloride in moderate yield,[10] and inadvertently in good yield by the in situ trapping of an allyl-protecting group during its removal.[11]

As part of our research into the synthesis of modified nucleobases for the preparation of novel peptide nucleic acids (PNAs), we discovered that protection of the exocyclic N2 amino group in the guanine precursor 2-amino-6-chloropurine as its Boc carbamate concomitantly activated that group as an acidic nucleophile (NuH) in the Mitsunobu reaction.[12] It was considered that the

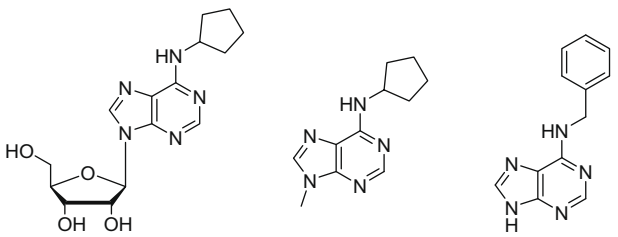

Figure 1. A potent and selective agonist (**1**) and antagonist (**2**) of the A_1 adenosine receptor, and a synthetic cytokinin (**3**).

* Tel.: +1 410 706 6361; fax: +1 410 706 5017.
E-mail address: sfletche@rx.umaryland.edu

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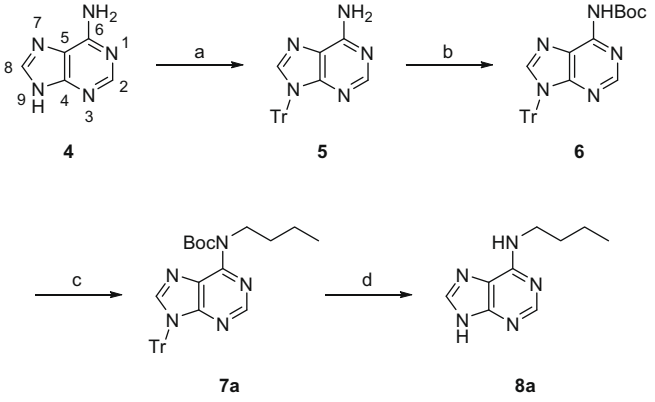
doi:10.1016/j.tetlet.2010.03.103

exocyclic N6 amino group of adenine might also be similarly activated, and, thereby, provide a novel route to N^6-substituted adenines. In addition, through the use of appropriate hydroxyl-protecting groups, we hoped to be able to apply this chemistry to the synthesis of N^6-substituted adenosines.

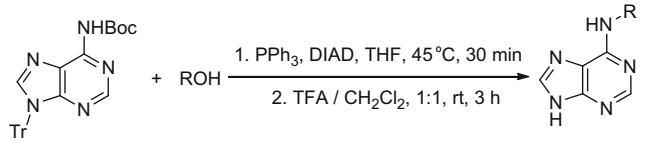
Towards this end, and as shown in Scheme 1, the requisite purine **6** was prepared from adenine (**4**) in two steps and in a very good overall yield (84%); simple N^9-tritylation of **4** gave **5**,[13] and then the exocyclic amino group was primed for the Mitsunobu reaction by a one-pot bis-Boc-protection and subsequent selective cleavage of one of the Boc groups with basic methanol at reflux (the reaction should be closely monitored as removal of the second Boc group slowly occurs under these conditions).[14] After optimization,[15] it was ascertained that the Mitsunobu reaction on the acidic NH of purine **6** could be effected with 1.5 equiv of each of 1-butanol, PPh_3 and di*iso*propyl azodicarboxylate (DIAD) in 30 min at 45 °C to afford the N^6-butylated compound **7a** in 94% yield. Only a trace amount of an unidentified, UV-active and more polar by-product (TLC, silica gel), presumably the N^1-butyl regioisomer, could be detected. These mild conditions are in contrast to those employed in the nucleophilic aromatic substitution reactions of 6-chloropurine with primary amines,[6] and may prove useful when using sensitive substrates. Subsequently, Boc and Tr deprotections were achieved by treatment with TFA to furnish **8a**, as the free base, in 97% yield after facile purification through a short pad of silica gel.

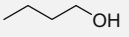
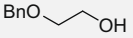
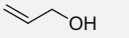
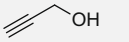
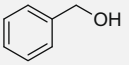
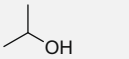
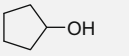
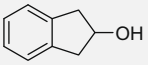
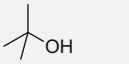
The optimized Mitsunobu conditions were applied to a series of alcohols, and the yields for these reactions are presented in Table 1 (the Boc and Tr deprotection reactions were ⩾95% in each case). Benzylic, allylic and propargylic alcohols coupled very well to afford the corresponding purines **7**, although the highest yields were reserved for the primary and secondary aliphatic alcohols. As anticipated, *tert*-butanol did not react under these conditions, due to steric hindrance at the tertiary carbon. After Boc and Tr deprotections, the target N^6-substituted adenines **8** were furnished as their free bases and as single tautomers, likely in the N^9–*H* form shown.[16] Significantly, to the best of my knowledge, this is the first example of the use of the Mitsunobu reaction to effect N^6-alkylation of adenine. Furthermore, it is the first detailed study of Mitsunobu-mediated alkylation of the exocyclic nitrogen of aromatic amidines in general, including 2-aminopyridines and 2-aminopyrimidines. It is anticipated that analogous transformations of such systems should also be possible with the chemistry reported herein.



Scheme 1. Reagents and conditions: (a) TrCl, DMF/pyridine, 3:1, rt, 7 h, 95%; (b) (1) Boc_2O, cat. DMAP, THF, rt, 12 h, 97%, (2) K_2CO_3, MeOH, reflux, 45 min, 91%; (c) (1) 1-butanol, PPh_3, THF, 45 °C, 2 min, (2) DIAD, 45 °C, 30 min, 94%; (d) TFA/CH_2Cl_2, 1:1, rt, 3 h, 97%.

Table 1
Reaction substrate scope in the N6 Mitsunobu coupling of purine **6** with ROH alcohols[a]



Entry	ROH	Product	Yield[b] (%)
1		**8a**	91
2		**8b**	89
3		**8c**	82
4		**8d**	81
5		**8e**	82
6		**8f**	88
7		**8g**	86
8[c]		**8h**	90
9		**8i**	0

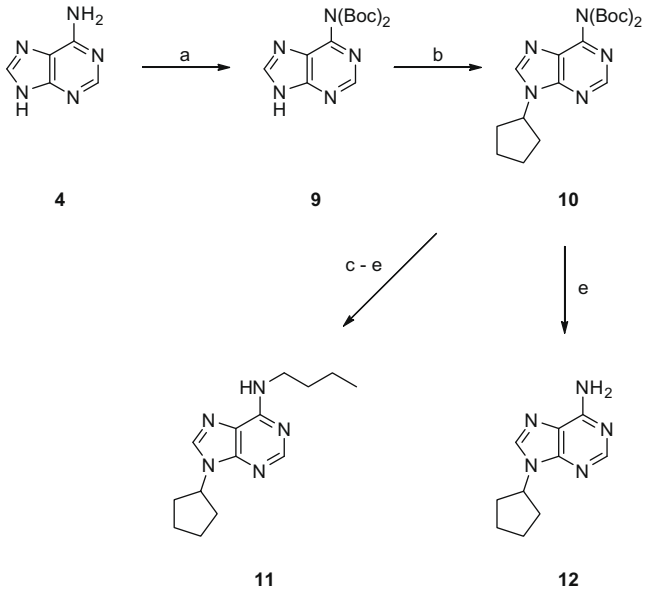
[a] Reaction conditions: (1) purine **6** (0.5 mmol), ROH (0.75 mmol) and PPh_3 (0.75 mmol) were dissolved in anhydrous THF (7 mL) at rt. After 2 min at 45 °C, DIAD (0.75 mmol) was added dropwise (over 30 s), and the reaction was stirred for 30 min at 45 °C.[17] (2) Purines **7** (0.25 mmol) were stirred in a 1:1 mixture of TFA/CH_2Cl_2 (5 mL) at rt for 3 h.

[b] Isolated overall yield for two steps.

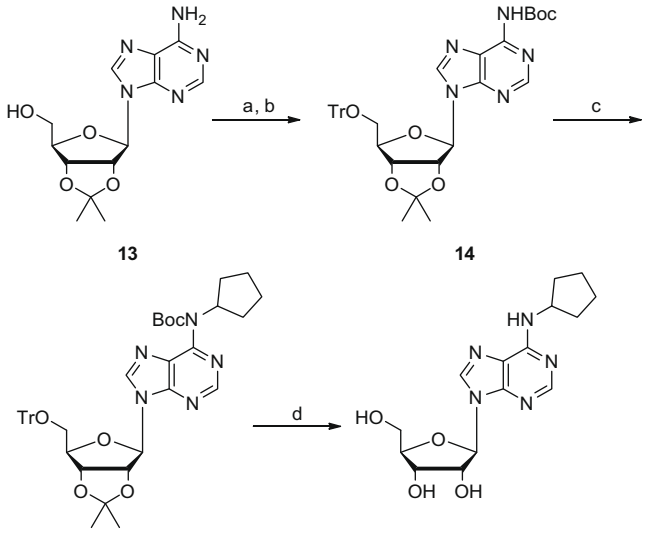
[c] 2.5 equiv of each of 2-indanol, PPh_3 and DIAD were required to consume all of purine **6**.

A high-yielding and regioselective approach to N^9-substituted adenines involves the use of the N^6,N^6-di-Boc-protected compound **9**[18] in the Mitsunobu reaction (Scheme 2).[19] Upon the selective mono-Boc deprotection of intermediate **10** as before, the chemistry presented herein may also be applied to the subsequent purine to furnish, after Boc deprotection, N^6,N^9-di-substituted adenine **11**. Alternatively, purine **10** may be directly deprotected with TFA to afford the corresponding N^9-substituted adenine **12**. This offers a convenient and efficient route to both N^9-mono- and N^6,N^9-di-substituted adenines from a common intermediate, and may prove useful in expediting access to A_1 adenosine receptor antagonists,[3] for example. As anticipated, starting from commercially available **13**, this chemistry is also suitable for the synthesis of N^6-substituted adenosines, including the potent and selective A_1 agonist N^6-cyclopentyladenosine[20] (**1**; Scheme 3), further demonstrating the utility of the Mitsunobu reaction in the functionalization of this purine nucleus.

In conclusion, a simple, efficient and mild approach towards the regioselective alkylation of the exocyclic N6 amino group of adenine and adenosine has been presented; by activating the amino group as its Boc carbamate, the N^6–NH becomes sufficiently acidic to participate in the Mitsunobu reaction. It is speculated that this chemistry will be directly applicable to aromatic amidines in general. Significantly, this mild approach avoids the relatively harsh conditions often required in the nucleophilic aromatic substitution reactions of primary amines with 6-chloropurines. Furthermore, in every case the key N^6-alkylation step was complete within 30 min, as opposed to the lengthy reaction times required for the corresponding nucleophilic aromatic substitution reaction. A variety of



Scheme 2. Reagents and conditions: (a) (1) Boc_2O, cat. DMAP, THF, rt, 16 h, (2) saturated $NaHCO_3$, MeOH, 50 °C, 1 h, 92%; (b) (1) cyclopentanol, PPh_3, THF, rt, 2 min, (2) DIAD, rt, 30 min, 94%; (c) K_2CO_3, MeOH, reflux, 45 min, 90%; (d) (1) 1-butanol, PPh_3, THF, 45 °C, 2 min, (2) DIAD, 45 °C, 30 min, 92%; (e) TFA/CH_2Cl_2, 1:1, rt, 1 h, 98% (for **11**), 99% (for **12**).



Scheme 3. Reagents and conditions: (a) TrCl, pyridine, 60 °C, 12 h, 87%; (b) (1) Boc_2O, cat. DMAP, THF, rt, 16 h, (2) K_2CO_3, MeOH, reflux, 45 min, 88%; (c) (1) cyclopentanol, PPh_3, THF, 45 °C, 2 min, (2) DIAD, 45 °C, 30 min, 93%; (d) 20% aq AcOH/THF, 5:1, 55 °C, 3 d, 85%.

alcohols were surveyed, and it was discovered that primary and secondary aliphatic alcohols coupled in excellent yields, slightly better than the more reactive benzylic, allylic and propargylic alcohols. Finally, the utility of this chemistry was further demonstrated by facilitating the rapid access to both N^9-mono- and N^6,N^9-disubstituted adenines from a common intermediate, as well as by the synthesis of the potent and selective A_1 adenosine receptor agonist N^6-cyclopentyladenosine (**1**).

Acknowledgement

The author gratefully acknowledges financial support for this work from the University of Maryland School of Pharmacy.

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14. Darnbrough, S.; Mervic, M.; Condon, S. M.; Burns, C. J. *Synth. Commun.* **2001**, *31*, 3273–3280.
15. *Typical procedure for Mitsunobu reaction:* A solution of purine **6** (239 mg, 0.5 mmol, 1 equiv), 1-butanol (69 μl, 0.75 mmol, 1.5 equiv) and PPh_3 (197 mg, 0.75 mmol, 1.5 equiv) in anhydrous THF (7 mL) was stirred at 45 °C for 2 min. Then, DIAD (148 μl, 0.75 mmol, 1.5 equiv) was added over about 30 s. The reaction mixture was stirred at 45 °C for 30 min under an N_2 atmosphere, after which time TLC analysis confirmed that the reaction was complete. The solvent was concentrated under reduced pressure. The residue was adsorbed onto silica gel from CH_2Cl_2, in the cold (<25 °C) to avert any Tr cleavage, and purified by flash column chromatography (eluent: CH_2Cl_2/Hex/EtOAc, 3:6:1) to give purine **7a** as a white foam (251 mg, 94%): δ_H ($CDCl_3$, 500 MHz) 0.90 (t, J = 7.4 Hz, 3H, CH_3), 1.34 (app sextet, J = 7.4 Hz, 2H, CH_2CH_3), 1.49 (s, 9H, $C(CH_3)$), 1.70 (app quintet, J = 7.4 Hz, 2H, $CH_2CH_2CH_3$), 3.98 (t, J = 7.4 Hz, 2H, N^6–CH_2), 7.15–7.20 (m, 6H, Tr), 7.28–7.34 (m, 9H, Tr), 7.98 (s, 1H, H8), 8.45 (s, 1H, H2); LRMS (ESI) m/z 533.9 (M+H).
16. *Typical procedure for Boc and Tr deprotections:* Purine **7a** (133 mg, 0.25 mmol) was dissolved in CH_2Cl_2 (2.5 mL), and then TFA (2.5 mL) was added. The reaction mixture was stirred for 3 h, by which time TLC showed that the deprotections were complete. All solvents were removed in vacuo, with residual TFA removed by co-evaporation with $CHCl_3$. The residue was adsorbed onto silica gel from CH_2Cl_2, then passed through a short pad of silica gel (eluent: CH_2Cl_2/MeOH/NH_4OH, 92:7:1) to furnish purine **8a** (46 mg, 97%) as its free base and as a white solid: δ_H (DMSO-d_6, 500 MHz) 0.90 (t, J = 7.4 Hz, 3H, CH_3), 1.32 (app sextet, J = 7.4 Hz, 2H, CH_2CH_3), 1.58 (app quintet, J = 7.4 Hz, 2H, $CH_2CH_2CH_3$), 3.47 (m, 1H, N^6–CH_2), 7.58 (br m, 1H, N^6–NH), 8.07 (s, 1H, H8) 8.17 (s, 1H, H2), 12.85 (br s, 1H, N^9–NH); LRMS (ESI) m/z 192.3 (M+H).
17. Purification of intermediate purines **7** was achieved by silica gel flash column chromatography, eluting with Hex/EtOAc, 2:1 or CH_2Cl_2/Hex/EtOAc, 3:6:1. Intermediate purines **7d**, **7f** and **7h** could not be completely purified by this method, being contaminated with varying amounts of DIAD-related by-products. Nevertheless, complete purification of the target purines **8d**, **8f** and **8h** was achieved after Boc and Tr removal in the overall yields shown (two steps) in Table 1.
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20. *Physical characterization data consistent with proposed structure of* **1**: δ_H (DMSO-d_6, 500 MHz) 1.50–1.64 (m, 4H, 4CH (cyclopentyl)), 1.67 – 1.74 (m, 2H, 2 CH (cyclopentyl)), 1.90–1.98 (m, 2H, 2CH (cyclopentyl)), 3.52–3.58 (m, 1H, H5′), 3.64–3.70 (m, 1H, H5′), 3.96 (dd, J = 8, 5 Hz, 1H, H4′), 4.14 (dd, J = 8, 5 Hz, 1H, H3'), 4.52 (m, 1H, $CH(CH_2)_2$ (cyclopentyl)), 4.60 (dd, J = 11, 6 Hz, 1H, H2′), 5.17 (d, J = 5 Hz, 1H, OH), 5.39–5.44 (m, 2H, 2 OH), 5.88 (d, J = 6 Hz, 1H, H1′), 7.76 (br m, 1H, NH), 8.19 (s, 1H, H8), 8.34 (s, 1H, H2); LRMS (ESI) m/z 336.2 (M+H).