ORGANIC LETTERS

2012
Vol. 14, No. 10
2488–2491

Efficient Synthesis of Exo-*N*-carbamoyl Nucleosides: Application to the Synthesis of Phosphoramidate Prodrugs

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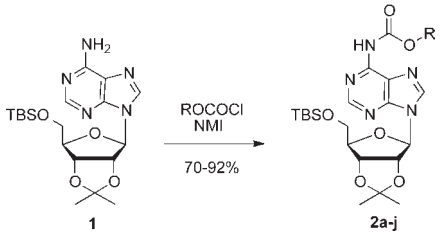
Received March 26, 2012

ABSTRACT



An efficient protection protocol for the 6-*exo*-amino group of purine nucleosides with various chloroformates was developed utilizing *N*-methylimidazole (NMI). The reaction of an *exo*-N^6-group of adenosine analogue 1 with alkyl/and aryl chloroformates under optimized conditions provided the N^6-carbamoyl adenosines (2a−j) in good to excellent yields. The reaction of N^6-Cbz-protected nucleosides (5a−c) with phenyl phosphoryl chloride (7) using *t*-BuMgCl followed by catalytic hydrogenation afforded the corresponding phosphoramidate pronucleotides (8a−c) in excellent yield.

Efficient introduction of protecting groups on polar and/or reactive functional groups is one of the most fundamental and critical aspects of modern organic synthesis.[1] For the synthesis of modified nucleosides and nucleotides, the chemoselective protection and subsequent deprotection of polar amino and hydroxyl groups has been integral in the preparation of biologically important analogues.[2] As part of our nucleoside discovery program, we sought a protection/deprotection strategy that would facilitate higher overall yields for the synthesis of phosphoramidate prodrugs.[3] Phosphoramidate prodrugs have been shown to enhance biological activity of parent nucleosides by increasing the intracellular nucleoside 5′-triphosphate levels via improved intracellular transport and/or bypassing the rate-limiting monophosphorylation step. Typical yields for the formation of phosphoramidate prodrugs with unprotected nucleosides are often single digit and often complicated by difficult isolation. A review of the literature for a potential protective group of *exo*-amino and/or hydroxyl groups on nucleosides revealed alkyl/aryloxycarbonyl groups as possible candidates for preparing phosphoramidate pronucleotides. Existing possible protecting

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10.1021/ol300777p
Published on Web 05/03/2012

groups from this class include trichloro-*tert*-butoxycarbonyl (TcBoc),[4] *tert*-butoxycarbonyl (Boc),[5] allyloxycarbonyl (Alloc),[6] phenyloxycarbonyl,[7] ethyloxycarbonyl,[8] β-cyanoethyloxycarbonyl,[9] 9-fluorenyloxycarbonyl (Fmoc),[10] 2-(trimethylsilyl)ethoxycarbonyl (Teoc),[11] and benzyloxycarbonyl (Cbz)[12] groups. While phosphoramidates are known to be somewhat stable to strongly acidic conditions,[13] our internal programs have shown phosphoramidates to have little to no stability to strongly basic and nucleophilic conditions (such as NH_3/MeOH, NH_4OH, piperazine, and NaOMe). The advantages of the mild removal conditions and orthogonality prompted us to consider Cbz as a useful protecting group of purine amino groups to prepare phosphoramidates. Although our previous efforts have shown that phosphoramidates are completely stable to catalytic hydrogenation,[14] introduction of a benzyloxycarbonyl (Cbz) group to the N^6-amino group of a purine nucleoside has been reported to be difficult, low yielding, and often requiring strong base[15] or preparation of a powerful Cbz transfer agent.[12a]

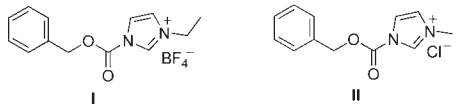
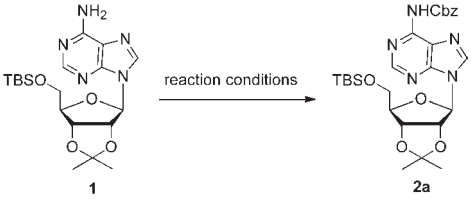


Figure 1. Benzyloxycarbonyl *N*-alkylimidazolium salts.

(1-((Benzyloxy)carbonyl)-3-methylimidazolium tetrafluoroborate (Rapoport's reagent), **I** (Figure 1), is the most widely utilized reagent for the protection of the *exo*-N^6-group of purines with Cbz.[12a] However, it is somewhat unstable, not commercially available, and must be prepared by a two-step sequence immediately prior to use.[16] As a part of our continuing studies, we herein report a more convenient Cbz-transfer method for *exo*-N^6-group on purine analogues and its application to an efficient synthetic route to 5′-phosphoramidates utilizing protection/deprotection of a 6-*N*-Cbz group.

Table 1. Result of Protection Reaction of N^6-Cbz-adenosine (**2a**)



	reaction conditions				
entry	CbzCl (equiv)	base (equiv)	solvent	temp (°C), time (h)	yield of **2a**[a] (%)
1	3.0	pyridine	pyridine	0 then rt, 72	[b]
2	3.0	Et_3N (4.0)	pyridine	0 then rt, 24	12
3	3.0	DMAP (4.0)	pyridine	0 then rt, 48	[b]
4	3.0	Im[c] (4.0)	CH_2Cl_2	0 then rt, 48	[b]
5	3.0	Et_3N/Im (3.0/6.0)	CH_2Cl_2	0 then rt, 72	[b]
6	3.0	DMAP/Im (3.0/6.0)	CH_2Cl_2	0 then rt, 48	[b]
7	3.0	DMAP/Im (3.0/6.0)	THF	0 then rt, 24	[b]
8	3.0	Et_3N (6.0)	CH_2Cl_2	0 then rt, 72	[b]
9	3.0	DMAP (6.0)	CH_2Cl_2	0 then rt, 72	[b]
10	3.0	NMI (6.0)	DMF	0 then rt, 24	34
11	3.0	NMI (6.0)	CH_3CN	0 then rt, 24	56
12	3.0	NMI (6.0)	THF	0 then rt, 24	45
13	3.0	NMI (6.0)	CH_2Cl_2	0 then rt, 24	**80**
14	2.0	NMI (4.0)	CH_2Cl_2	0 then rt, 48	**72**
15	4.0	NMI (8.0)	CH_2Cl_2	0 then rt, 12	**89**

[a] Isolated yield from silica gel chromatography. [b] A trace amount of **2a** was detected by LC/MS analysis along with starting material **1**. [c] Im = imidazole.

Initially, we chose to investigate the reaction of an *exo*-N^6-group of adenosine derivative (**1**)[17] with CbzCl using previously reported conditions.[14,18] The reaction of **1** with CbzCl in the presence of a variety of organic bases such as

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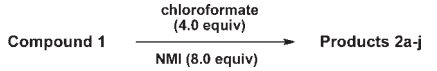
pyridine, Et_3N, DMAP, and imidazole in several different solvents gave N^6-Cbz-adenosine derivative (**2a**) in yields that ranged from trace to 12% as summarized in Table 1 (entries 1−9). When *N*-methylimidazole (NMI) was used, the yield of **2a** was significantly improved to 34−80% depending on the solvent (DMF; 34%, CH_3CN; 56%, THF; 45%, CH_2Cl_2; 72−80%, entries 10−14). The optimal yield of **2a** (89%) was obtained with 4 equiv of CbzCl in the presence of 8 equiv of NMI in CH_2Cl_2 for 12 h at room temperature (Table 1, entry 15). We postulate the improvement in yield with NMI is due to transient formation of benzyloxycarbonyl *N*-methylimidazolium chloride, **II**, similar to Rapoport's reagent, **I** (Figure 1).

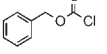
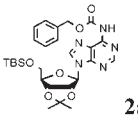
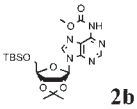
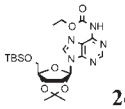
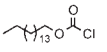
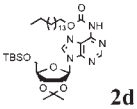
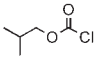
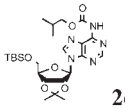
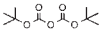
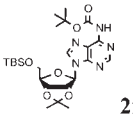
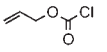
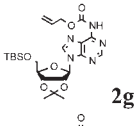
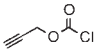
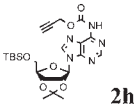
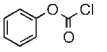
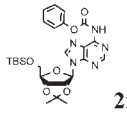
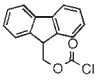
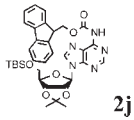
To determine the generality of these conditions, we applied these conditions to the reaction of the *exo*-N^6-group on adenosine derivative **1** with a variety of chloroformate analogues. The reaction of **1** with methyl, ethyl, hexadecyl, and isobutyl chloroformates gave N^6-carbamoyl adenosines (**2b**−**e**) in high yields as shown in Table 2 (75−92%, entries 2−5). However, the reaction of **1** with *tert*-butoxycarbonyl anhydride ($(Boc)_2O$) gave the corresponding N^6-Boc-adennosine **2f** in a disappointing 14% yield along with N^6,N^6-bis-Boc-adenosine (**9**) in 25% yield[19] (Table 2, entry 6). The combination of $(Boc)_2O$ with NMI was less efficient than $(Boc)_2O$/DMAP in THF for the protection of *exo*-amino groups of this nucleoside analogue.[5a] The reaction of **1** with *tert*-butyl chloroformate was not attempted as this reagent has limited stability and is therefore not widely available or utilized. The protection of **1** with allyl, propargyl, phenyl and Fmoc chloroformates provided their corresponding carbamoyl derivatives (**2g**−**j**) in good yields (70−82%, Table 2, entries 7−10).

Further, we examined Cbz-protected *N*-/*O*-groups of purine nucleosides in order to apply this method to prepare aryl phosphoramidates. The synthesis of most phosphoramidates is plagued by low to moderate yields due to both poor chemoselectivity in the phosphoramidate-forming reaction and/or poor solubility of the nucleoside in applicable solvents.[20] Many of the currently utilized protocols for the synthesis of protected phosphoramidates involve acidic deprotection conditions under which the relative instability of phosphoramidates leads to moderate yields.[13,17c]

As an extension of our high yielding approach to phosphoramidates of pyrimidine nucleosides involving Cbz protection of the sugar hydroxyl groups,[14] we applied our more forcing Cbz-transferring conditions to the preparation of phenyl phosphoramidates of adenosine (A), guanosine (G), and 2,6-diaminopurine ribose (DAPR), as shown in Scheme 1. The Cbz group was introduced in high yield on N^6 and 2′,3′-*O*-positions of 5′-TBS protected A analogue **3a** and DAPR analogue **3c**. For the G analogue, **3b**, only the 2′,3′-*O*-positions were acylated. Even under the most forcing CbzCl/NMI conditions we could not detect acylation of the 6-*O* position of the ribo G nucleoside. In addition, although an excess of CbzCl/NMI (as much as 6.0/12.0 equiv) was employed in this protection sequence we did not observe any N^2-amino acylation of A, G, or DAPR analogues.

Table 2. Reaction of **1** with Various Chloroformates



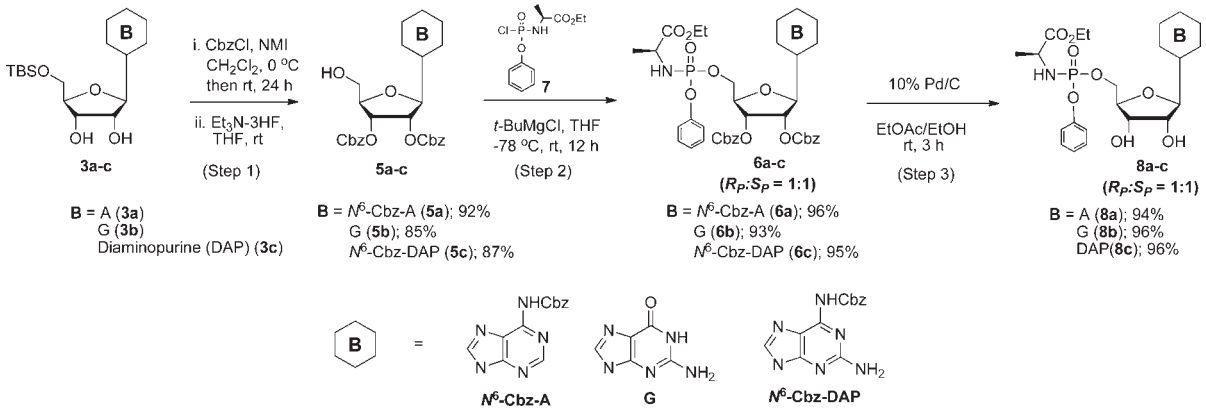
entry	protecting reagent	time (h)	product (**2a-j**)	yield (%)[a]
1		12	 **2a**	89
2		12	 **2b**	75
3		12	 **2c**	86
4		12	 **2d**	92
5		24	 **2e**	88
6		24	 **2f**	14[b]
7		12	 **2g**	82
8		24	 **2h**	76
9		12	 **2i**	70
10		12	 **2j**	78

[a] Isolated yield by silica gel column. [b] Major product was N^6,N'^6-bis-Boc-adenosine derivative (**9**) in 25% yield.[19]

(19) Data and experimental conditions for all compounds are available in the Supporting Information.

(20) (a) McGuigan, C.; Pathirana, R. N.; Mahmood, N.; Hay, A. J. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 701–704. (b) Kumar, D.; Mamiya, B. M.; Kern, J. T.; Kerwin, S. M. *Tetrahedron Lett.* **2001**, *42*, 565–567. (c) Sofia, M. J.; Bao, D.; Chang, W.; Du, J.; Nagarathnam, D.; Rachakonda, S.; Reddy, P. G.; Ross, B. S.; Wang, P.; Zhang, H.-R.; Bansal, S.; Espiritu, C.; Keilman, M.; Lam, A. M.; Steuer, H. M. M.; Niu, C.; Otto, M. J.; Furman, P. A. *J. Med. Chem.* **2010**, *53*, 7202–7218. (d) Chang, W.; Bao, D.; Chun, B.-K.; Naduthambi, D.; Nagarathnam, D.; Rachakonda, S.; Reddy, P. G.; Ross, B. S.; Zhang, H.-R.; Bansal, S.; Espiritu, C. L.; Keilman, M.; Lam, A. M.; Niu, C.; Steuer, H. M.; Furman, P. A.; Otto, M.; Sofia, M. J. *J. Med. Chem. Lett.* **2011**, *2*, 130–135.

Scheme 1. Synthesis of Purine Phenyl Phosphoramidates (**8a**–**c**)[a]



[a] Product was a diastereomeric mixture in which the R_P/S_P or S_P/R_P ratio ranged from 1:1 to 1:1.5.

Treatment with Et_3N–3HF provided Cbz-protected nucleosides **5a**–**c** in excellent yield (Scheme 1). In the case of DAPR, in addition to the 87% yield of desired N^6,2′,3′-*O*-tris-Cbz-DAPR, **5c**, N^6,N^6,2′,3′-*O*-tetra-Cbz-DAPR, **10** was obtained in 5% yield.[19] Subsequently, the reaction of protected nucleosides **5a**–**c** with the phosphoryl chloride **7** in the presence of *t*-BuMgCl in THF gave Cbz-protected phosphoramidates **6a**–**c** as a R_P/S_P or S_P/R_P diastereomeric mixture (ranged from 1:1 to 1:1.5 by ^{1}H NMR) in excellent yields without any detectable cleavage of *N*- or *O*-Cbz bonds at either chemical step. We also note that the tri-Cbz-protected C and di-Cbz-protected U previously reported[14] when reacted with **7** in the presence of *t*-BuMgCl in THF gave Cbz-protected phosphoramidates in 94% and 97%, respectively.[19] Removal of Cbz groups from the phosphoramidates **6a**–**c** with Pd/C and H_2 (1 atm) afforded **8a**–**c** in 94%, 96% and 96% yield, respectively.

In summary, an efficient CbzCl protection protocol for the N^6-amino of adenine and diaminopurine nucleosides which also acylated the hydroxyl groups of the ribose ring was successfully developed by utilizing NMI in CH_2Cl_2. This robust protocol does not require a two-step synthesis of a Cbz transfer agent and provides good to excellent yields under mild conditions. In addition, we successfully applied this method to a large variety of alkyl and aryl chloroformates to afford their corresponding exo-N^6-carbamoyl adenosines in good to excellent yields. The reaction of 6-*N*, 2′,3′-*O* protected A (**5a**), G (**5b**) and DAPR (**5c**) with phenyl(ethoxy-L-alaninyl)phosphorochloridate (**7**) provided the Cbz-protected phosphoramidates (**6a**–**c**) in excellent yield. Cbz group cleavage was not observed during the phosphoryl chloride coupling conditions of *t*-BuMgCl or NMI and also not observed during the Et_3N–3HF promoted desilylation. Overall, we have developed a highly efficient novel method for the synthesis of purine nucleoside phosphoramidates.

Acknowledgment. This work was supported in part by NIH Grant No. 5P30-AI-50409 (CFAR) and by the Department of Veterans Affairs.

Supporting Information Available. Experimental procedures, characterization data, and ^{1}H and ^{13}C NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare the following competing financial interest(s): Dr. Schinazi is the founder and a major shareholder of RFS Pharma, LLC. Emory received no funding from RFS Pharma, LLC to perform this work and vice versa.