

Published in final edited form as:

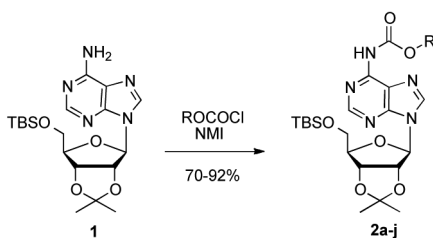
Org Lett. 2012 May 18; 14(10): 2488–2491. doi:10.1021/ol300777p.

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

Jong Hyun Cho, Steven J. Coats, and Raymond F. Schinazi*

Center for AIDS Research, Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, and Veterans Affairs Medical Center, Decatur, GA 30033, USA, and RFS Pharma, LLC, 1860 Montreal Road, Tucker, GA 30084, USA

Abstract



An efficient protection protocol for the 6-*exo*-amino group of purine nucleosides with various chloroformates was developed utilizing *N*-methyl imidazole (NMI). The reaction of an *exo-N*⁶-group of adenosine analog 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 biology important analogs.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 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:

*rschina@emory.edu.

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

(trimethylsilyl)ethoxycarbonyl (Teoc)¹¹ and benzyloxycarbonyl (Cbz)¹² groups. While phosphoramidates are known to be somewhat stable to strongly acid conditions¹³ our internal programs have shown phosphoramidates to have little to no stability to strongly basic and nucleophilic conditions (such as NH₃/MeOH, NH₄OH, piperazine and NaOMe). The advantages of the mild removal conditions and orthogonally 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 base¹⁵ or preparation of a powerful Cbz transfer agent.^{12a}

(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 analogs and its application to an efficient synthetic route to 5'-phosphoramidates utilizing protection/deprotection of a 6-*N*-Cbz group.

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 pyridine, Et₃N, 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*-methyl imidazole (NMI) was used, the yield of **2a** was significantly improved to 34-80% depending on the solvent (DMF; 34%, CH₃CN; 56%, THF; 45%, CH₂Cl₂; 72:80%, entries 10-14). The optimal yield of **2a** (89%) was obtained with four equivalents of CbzCl in the presence of eight equivalents of NMI in CH₂Cl₂ 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 analogs. 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 *t*-butoxycarbonyl anhydride ((Boc)₂O) gave the corresponding *N*⁶-Boc-adenosine **2f** in a disappointing 14% yield along with *N*⁶,*N*⁶-bis:Boc:adenosine (**9**) in 25% yield¹⁹ (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 analog.^{5a} The reaction of **1** with *t*-butyl chloroformate was not attempted as this reagent has limited stability and 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}

Treatment with Et₃N·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 ¹H 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 reported¹⁴ 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 H₂ (1 atm) afforded **8a-c** in 94%, 96% and 96% yield respectively.

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 analog **3a** and DAPR analog **3c**. For the G analog, **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 analogs.

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 CH₂Cl₂. 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 Et₃N:3HF promoted desilylation. Overall, we have developed a highly efficient novel method for the synthesis of purine nucleoside phosphoramidates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported in part by NIH grant 5P30-AI-50409 (CFAR), and by the Department of Veterans Affairs.

References

- (1). (a) Wuts, PGM.; Greene, TW. Green's Protective Groups in Organic Synthesis. 4th edition. John Wiley and Sons, Inc.; Hoboken, NJ: 2007. (b) Kocienski, PJ. Protecting Groups; Geoug Thieme Verlag Stuttgart. New York: 1994.
- (2). (a) Samano V, Miles RW, Robins MJ. J. Am. Chem. Soc. 1994; 116:9331–9332.(b) Miles RW, Samano V, Robins MJ. J. Am. Chem. Soc. 1995; 117:5951–5957.(c) Cui Z, Zhang L, Zhang B. Tetrahedron Lett. 2001; 42:561–563.(d) Nguyen-Trung NQ, Terenzi S, Scherer G, Strazewski P. Org. Lett. 2003; 5:2603–2606. [PubMed: 12868869] (e) Nowak I, Robins MJ. Org. Lett. 2003; 5:3345–3348. [PubMed: 12943423] (f) Zhong M, Nowak I, Robins MJ. J. Org. Chem. 2006;

- 71:7773–7779. [PubMed: 16995685] (g) Derudas M, Carta D, Brancale A, Vanpouille C, Lisco A, Margolis L, Balzarini J, McGuigan C. *J. Med. Chem.* 2009; 52:5520–5530. [PubMed: 19645484] (h) Arico JW, Calhoun AK, Salandria KJ, McLaughlin LW. *Org. Lett.* 2010; 12:120–122. [PubMed: 19961189]
- (3). For reviews: Bobeck DR, Coats SJ, Schinazi RF. *Antivir. Ther.* 2010; 15:935–950. [PubMed: 21041908] Erion MD, Hecker SJ. *J. Med. Chem.* 2008; 51:2328–2345. [PubMed: 18237108] Schultz C. *Bioorg. Med. Chem.* 2003; 11:885–898. [PubMed: 12614874] Mehellou Y, Balzarini J, McGuigan C. *Chem. Med. Chem.* 2009; 4:1779–1791. [PubMed: 19760699] Sofia MJ. *Antivir. Chem. Chemother.* 2011; 22:23–49. [PubMed: 21860070]
- (4). (a) Hotoda H, Saito R, Sekine M, Hata T. *Tetrahedron.* 1990; 46:1181–1190. (b) Strasser M, Ugi I. *Acta Chem. Scand.* 1993; 47:125–130.
- (5). (a) Dey S, Garner PJ. *Org. Chem.* 2000; 65:7697–7699. (b) Bazzanini R, Gouy M:H, Peyrottes S, Gosselin G, Perigaud C, Manfrendini S. *Nucleosides, Nucleotides & Nucleic acids.* 2005; 24:1635–1649. (c) Wojciechowski F, Hudson RHE. *J. Org. Chem.* 2008; 73:3807–3816. [PubMed: 18412392] (d) Wang R:W, Gold B. *Org. Lett.* 2009; 11:2465–2468. [PubMed: 19422208]
- (6). (a) Rigby JH, Moore TL, Rege S. *J. Org. Chem.* 1986; 51:2400–2402. (b) Wu W, Sigmund J, Peters GJ, Borch RF. *J. Med. Chem.* 2007; 50:3743–3746. [PubMed: 17602464]
- (7). (a) Anzai K, Hunt JB, Zon G. *J. Org. Chem.* 1982; 47:4281–4285. (b) Anzai K, Uzawa J. *J. Org. Chem.* 1984; 49:5076–5080.
- (8). Chheda GB, Hong CI. *J. Med. Chem.* 1971; 14:748–753. [PubMed: 5286530]
- (9). Chen T, Fu J, Greenberg MM. *Org. Lett.* 2000; 2:3691–3694. [PubMed: 11073677]
- (10). (a) Kutyanvin, IV.; Woo, J.; Lukhtanov, EA.; Meyer, RB., Jr.; Gamper, HB. WO1997012896. 1997. (b) Alvarado, GG. WO2004029297. 2004.
- (11). Ferreira F, Morvan F. *Nucleosides, Nucleotides & Nucleic acids.* 2005; 24:1009–1013.
- (12). (a) Watkins BE, Kiely JS, Rapoport H. *J. Am. Chem. Soc.* 1982; 104:5702–5708. (b) Liu F, Austin D. *J. Org. Lett.* 2001; 3:2273–2276. (c) Wandzik I, Bieg T, kadela M. *Nucleosides, Nucleotides & Nucleic acids.* 2008; 27:1250–1256.
- (13). (a) Perrone P, Daverio F, Valente R, Rajyaguru S, Martin JA, Lévêque V, Pogam SL, Najera I, Klumpp K, Smith DB, McGuigan C. *J. Med. Chem.* 2007; 50:5463–5470. [PubMed: 17914786] (b) Gardelli C, Attenni B, Donghi M, Meppen M, Pacini B, Harper S, Marco AD, Fiore F, Giuliano C, Pucci V, Laufer R, Gennari N, Marcucci I, Leone JF, Olsen DB, MacCoss M, Rowley M, Narjes F. *J. Med. Chem.* 2009; 52:5394–5407. [PubMed: 19725579] (c) Perlíková P, Pohl R, Votruba I, Shih R, Birkuš G, Cihlá T, Hocek M. *Bioorg. Med. Chem.* 2011; 19:229–242. [PubMed: 21134754]
- (14). Cho JH, Amblard F, Coats SJ, Schinazi RF. *Tetrahedron.* 2011; 67:5487–5493. [PubMed: 22993455]
- (15). (a) Azzena U, Dettori G, Pisano L, Pittalis M. *Syn. Comm.* 2007; 37:3623–3634. (b) Rosse G, Sequin U. *Helv. Chimica Acta.* 1997; 80:653–670. (c) Peifer M, De Giacomo F, Schandl M, Vasella A. *Helv. Chimica Acta.* 2009; 92:1134–1166. (d) Sumita Y, Shirato M, Ueno Y, Matsuda A, Shuto S. *Nucleosides, Nucleotides & Nucleic Acids.* 2000; 19:175–187.
- (16). For other Cbz transfer reagents applied to N⁶-purines see: Komiyama M, Aiba Y, Ishizuka T, Sumaoka J. *Nature Protocols.* 2008; 3:646–654. Schlama T, Manfre F. US20060149051. 2006 Rayan A, Falah M. US201000284959. 2010
- (17). (a) Homma H, Watanabe Y, Abiru T, Murayama T, Momura Y, Matsuda A. *J. Med. Chem.* 1992; 35:2881–2890. [PubMed: 1495019] (b) Cho JH, Bernard DL, Sidwell RW, Kern ER, Chu CK. *J. Med. Chem.* 2006; 49:1140–1148. [PubMed: 16451078] (c) McGuigan C, Gilles A, Madela K, Aljarah M, Holl S, Jones S, Vernachio J, Hutchins J, Bryant KD, Gorovits E, Ganguly B, Hunley D, Hall A, Kolykhalov A, Liu Y, Muhammad J, Raja N, Walters R, Wang J, Chamberlain S, Henson G. *J. Med. Chem.* 2010; 53:4949–4957. [PubMed: 20527890]
- (18). Johnson DC II, Widlanski TS. *Org. Lett.* 2004; 6:4643–4646. [PubMed: 15575650]
- (19). Data and experimental conditions for all compounds are available in Supporting Information.
- (20). (a) McGuigan C, Pathirana RN, Mahmood N, Hay AJ. *Bioorg. Med. Chem. Lett.* 1992; 2:701–704. (b) Kumar D, Mamiya BM, Kern JT, Kerwin SM. *Tetrahedron Lett.* 2001; 42:565–567. (c)

Sofia MJ, Bao D, Chang W, Du J, Nagarathnam D, Rachakonda S, Reddy PG, Ross BS, Wang P, Zhang H-R, Bansal S, Espiritu C, Keilman M, Lam AM, Steuer HMM, Niu C, Otto MJ, Furman PA. *J. Med. Chem.* 2010; 53:7202–7218. [PubMed: 20845908] (d) Chang W, Bao D, Chun B:K, Naduthambi D, Nagarathnam D, Rachakonda S, Reddy PG, Ross BS, Zhang H-R, Bansal S, Espiritu CL, Keilman M, Lam AM, Niu C, Steuer HM, Furman PA, Otto M, Sofia MJ. *J. Med. Chem. Lett.* 2011; 2:130–135.

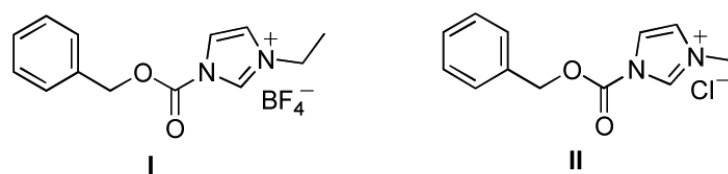
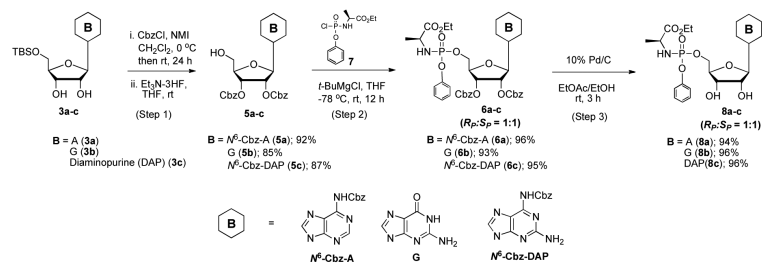


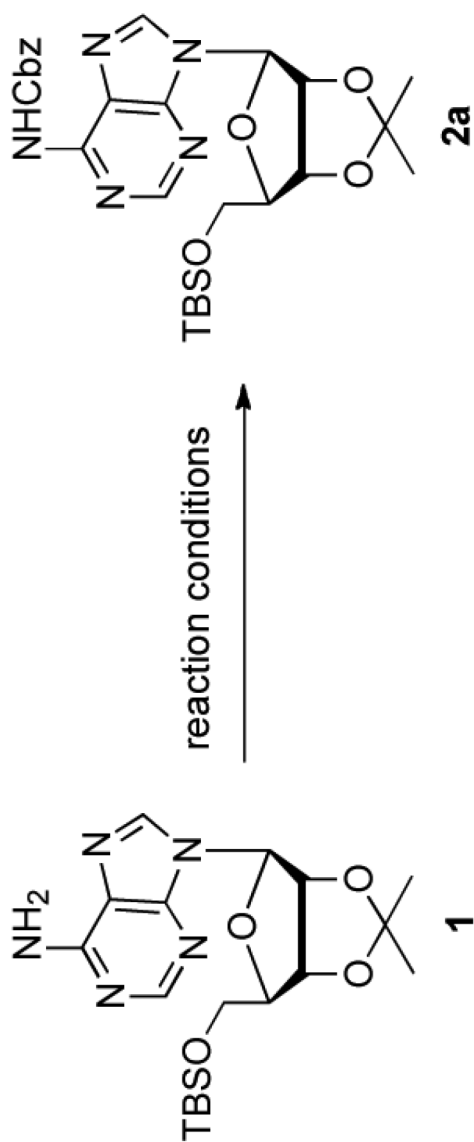
Figure 1.
Benzyloxycarbonyl N-alkylimidazolium salts

**Scheme 1.**

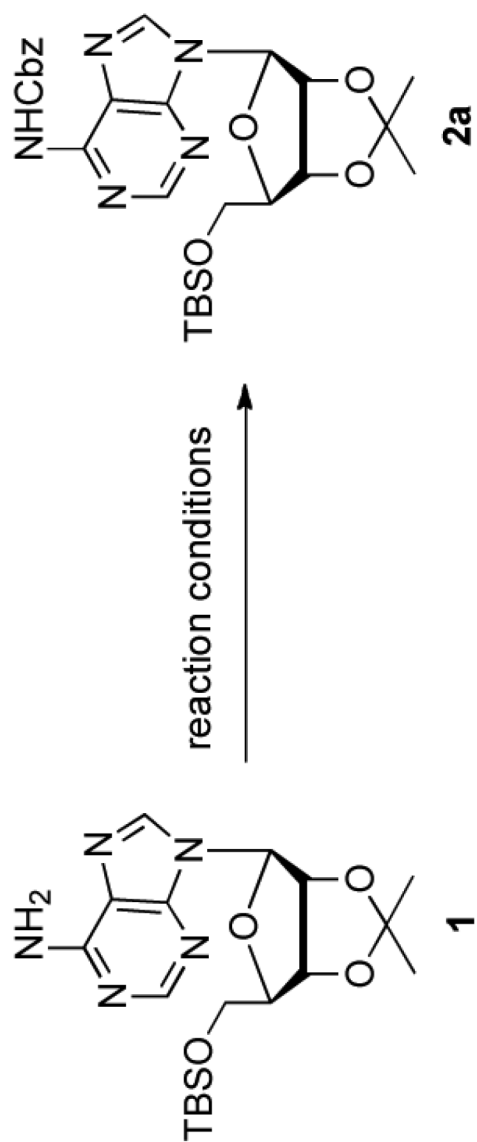
Synthesis of purine phenyl phosphoramidates (**8a-c**)^a

^aProduct was a diastereomeric mixture in which R_P/S_P or S_P/R_P ratio ranged from 1:1 to 1:1.5.

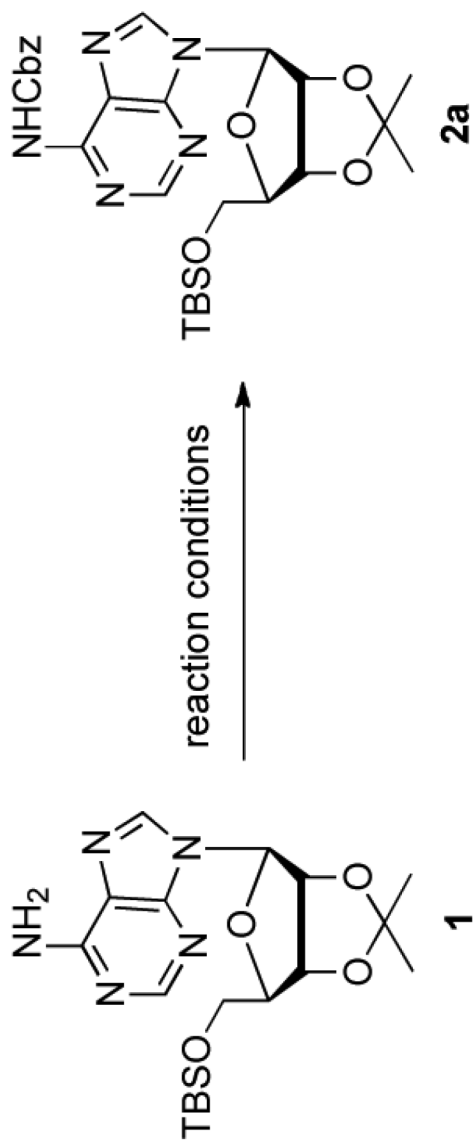
Table 1

Result of protection reaction of *N*⁶-Cbz-adenosine (**2a**)

entry	Reaction conditions			temp /time	yield of 2a (%) ^a
	CbzCl (equiv)	base (equiv)	solvent		
1	3.0	Pyridine	Pyridine	0 °C then rt, 72 h	-- ^b
2	3.0	Et ₃ N (4.0)	Pyridine	0 °C then rt, 24 h	12
3	3.0	DMAP (4.0)	Pyridine	0 °C then rt, 48 h	-- ^b
4	3.0	Im ^c (4.0)	CH ₂ Cl ₂	0 °C then rt, 48 h	-- ^b
5	3.0	Et ₃ N/Im (3.0/6.0)	CH ₂ Cl ₂	0 °C then rt, 72 h	-- ^b
6	3.0	DMAP/Im (3.0/6.0)	CH ₂ Cl ₂	0 °C then rt, 48 h	-- ^b



entry	Reaction conditions				yield of 2a (%) ^a
	ChzCl (equiv)	base (equiv)	solvent	temp /time	
7	3.0	DMAP/ Im (3.0/6.0)	THF	0 °C then rt, 24 h	-- ^b
8	3.0	Et ₃ N (6.0)	CH ₂ Cl ₂	0 °C then rt, 72 h	-- ^b
9	3.0	DMAP (6.0)	CH ₂ Cl ₂	0 °C then rt, 72 h	-- ^b
10	3.0	NMI (6.0)	DMF	0 °C then rt, 24 h	34
11	3.0	NMI (6.0)	CH ₃ CN	0 °C then rt, 24 h	56
12	3.0	NMI (6.0)	THF	0 °C then rt, 24 h	45
13	3.0	NMI (6.0)	CH ₂ Cl ₂	0 °C then rt, 24 h	80
14	2.0	NMI (4.0)	CH ₂ Cl ₂	0 °C	72



entry	Reaction conditions				yield of 2a (%) ^a
	ChzCl (equiv)	base (equiv)	solvent	temp /time	
15	4.0	NMI (8.0)	CH ₂ Cl ₂	then rt, 48 h 0 °C then rt, 12 h	89

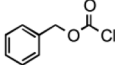
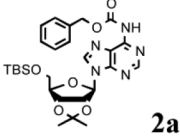
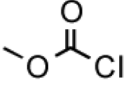
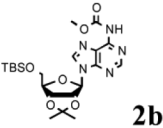
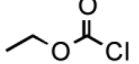
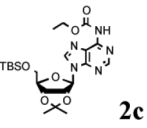
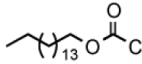
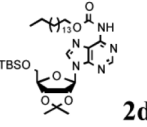
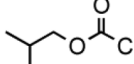
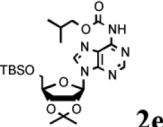
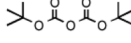
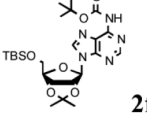
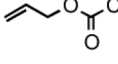
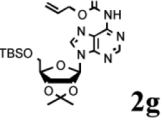
^a Isolated yield from silica gel chromatography.

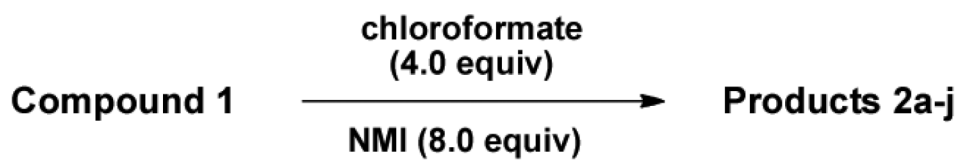
^b A trace amount of **2a** was detected by LC/MS analysis along with starting material **1**.

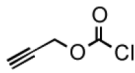
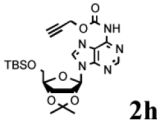
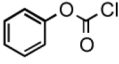
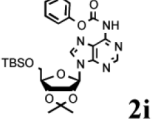
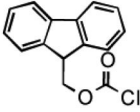
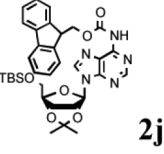
^c Im = imidazole.

Table 2

Reaction of **1** with various chloroformates

Compound 1		chloroformate (4.0 equiv)		Products 2a-j	
		NMI (8.0 equiv)			
entry	protecting reagent	time (h)	product (2a-j)	yield (%) ^a	
1		12		89	
2		12		75	
3		12		86	
4		12		92	
5		24		88	
6		24		14 ^b	
7		12		82	



entry	protecting reagent	time (h)	product (2a-j)	yield (%) ^a
8		24	 2h	76
9		12	 2i	70
10		12	 2j	78

^aIsolated yield by silica gel column.

^bMajor product was N⁶,N⁶-bis-Boc-adenosine derivative (**9**) in 25% yield.¹⁹