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Solventless Protocol for Efficient Bis-*N*-Boc Protection of Adenosine, Cytidine, and Guanosine Derivatives

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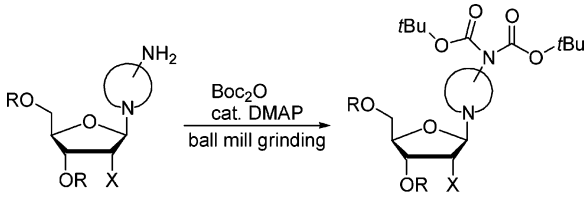

adenosine, cytidine, guanosine derivatives

A solvent-free reaction employing a simple low-energy ball mill apparatus converts the amino groups of adenosine, 2-deoxyadenosine, cytidine, 2-deoxycytidine, guanosine, and 2-deoxyguanosine as well as some of their ribosyl *O*-protected derivatives to the corresponding bis-*N*-Boc carbamates. In the case of guanosine compounds, the carbonyl group of the base moiety was also blocked as its *O*-Boc enol carbonate. A variation of this approach using transient in situ *O*-silylation permitted the preparation of bis-*N*-Boc nucleosides in which the sugar hydroxyls were unprotected. The ball mill reactions were rapid, convenient, and very high-yielding except in the case of the guanosine compounds. This highly efficient method protects the amino groups of these nucleosides with a base stable and acid labile group suitable for further synthetic manipulation.

Introduction

The synthesis of modified nucleosides and oligonucleotides requires the protection and deprotection of functional groups on both the sugar and base portions of the monomers. In addition to allowing regioselective reactions, protection enhances the generally poor organic solubility of the parent nucleosides. The amino groups in adenosine, cytidine, and guanosine are frequently protected by *N*-acylation or bis-*N*-acylation.[1,2] The alkaline conditions used to remove these protecting groups restrict the range of other functional and protecting groups that may be present in synthetic nucleosides. Carbamates and biscarbamates that can be removed under mildly basic to neutral conditions are more useful *N*-protecting groups for these nucleosides.[3,4,5] In contrast, acid-labile *N*-protecting groups for nucleosides have received little attention in the literature.

In the course of other research, we required 5′-amide derivatives of adenosine-5′-carboxylate **1**[6] but making these proved difficult because compound **1** was very poorly soluble in the organic solvents commonly used for amide formation. We thought that protecting the 6-amino group of **1** would enhance its solubility. *N*-Benzoylated nucleobases are more labile to base-promoted solvolysis than are other amides, but the literature also revealed that yields for these reactions could be variable. This did not encourage us to rely on reactivity differences between amides and amidines to obtain our proposed targets. However, the use of an acid-labile *N*-protecting group offered an alternative solution to this problem. The most common acid-labile *N*-protecting group in general use is the

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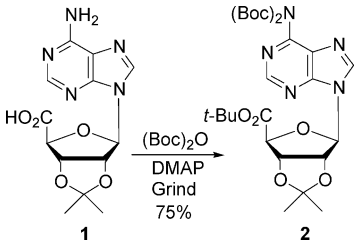
SCHEME 1. Solventless Reaction of 1 with $(Boc)_2O$ and DMAP



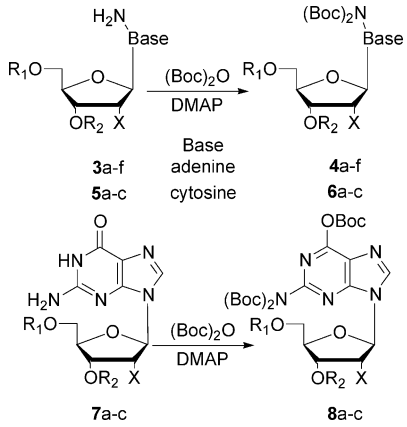
tert-butoxycarbamate (Boc) group. Boc groups have seldom been used for the protection of modified nucleosides.[7−9] Our attempts to protect the *N*-6 group of **1** with Boc using the usual methods were unsuccessful, and it was evident that the problems of solubility were a major factor in our difficulties.

Solvent-free synthesis is gaining popularity, especially in industry, because it may be both more environmentally benign and more economically feasible.[10] We therefore examined Boc protection of **1** in the absence of any solvent. To our satisfaction, grinding **1** with an excess of di-*tert*-butyl dicarbonate ($(Boc)_2O$) and 4-*N*,*N*-(dimethylamino)pyridine (DMAP) resulted in the rapid formation of a less-polar product, which proved to be *t*-butyl *N*,[6] *N*[6]-bis [(*tert*-butoxy)carbonyl]-2′,3′-*O*-isopropylidene-adenosine-5′-carboxylate **2** (Scheme 1). Bis-*N*-protected amines often avoid side reactions or unwanted cyclizations.[11] Solution-phase methods for making bis-*N*-Boc protected amines generally require the use of strong bases,[12] although some methods employing DMAP catalysis have been reported.[11] A few instances of bis-*N*-Boc protected adenosine and cytosine derivatives have been reported.[7a−9] We now report that solventless reactions of this kind provide rapid and efficient access to a variety of bis-*N*-Boc protected adenosine and cytidine derivatives. We also describe our results with guanosine compounds.

Results and Discussion

The reaction of amines with $(Boc)_2O$ cannot be performed in a closed glass flask or vial as $CO_2(g)$ is liberated during the reaction. Experiments in closed containers agitated in a shaker resulted in cracking because of excess pressure buildup. A simple ball mill apparatus consisting of a thick-walled round-bottom flask, 9 mm glass beads, and an overhead stirrer with a loosely fitting shaft proved to be very satisfactory for benchtop-scale reactions. This simple mill provided a relatively gentle grinding action, which was nevertheless quite adequate for these reactions. Ball milling has been a part of industrial materials processing for many years and has recently been recognized as a useful technique in organic synthesis.[13]

SCHEME 2. Solventless *N*-Protection of *O*-Protected Nucleoside Derivatives[a]



[a] See Table 1 for R- and X-groups, conditions, and yields.

TABLE 1. Solventless Reactions of Nucleoside Derivatives with $(Boc)_2O$ and DMAP

substrate	R_1	R_2	X	conditions[a]	product (yield %)
3a	Ac	Ac	OAc	A, 6 h	**4a** (90)
3b	TBDMS	TBDMS	OTBDMS	B, 2 h	**4b** (99)
3c	Ac	$C(Me)_2$	$OC(Me)_2$	B, 4 h	**4c** (96)
3d	TBDMS	$C(Me)_2$	$OC(Me)_2$	B, 2 h	**4d** (99)
3e	Ac	Ac	H	B, 2 h	**4e** (99)
3f	TBDMS	TBDMS	H	B, 1 h	**4f**[7a] (99)
5a	TBDMS	TBDMS	OTBDMS	C, 1 h	**6a** (99)
5b	TBDMS	TBDMS	H	C, 1 h	**6b** (99)
5c	Ac	Ac	H	C, 2 h	**6c** (50)
7a	Ac	Ac	OAc	D, 7 h	**8a** (25)
7b	TBDMS	TBDMS	OTBDMS	D, 6 h	**8b** (40)
7c	TBDMS	TBDMS	H	E, 6 h	**8c** (70)

[a] Reaction conditions: (Method A) 30 mol % DMAP, 4 eq $(Boc)_2O$; (Method B) 10 mol % DMAP, 3 equiv $(Boc)_2O$; (Method C) 20 mol % DMAP, 4 equiv $(Boc)_2O$; (Method D) 20 mol % DMAP, 5 equiv $(Boc)_2O$; (Method E) 40 mol % DMAP, 6 equiv $(Boc)_2O$.

To determine the scope of this solvent-free reaction, we applied it to common *O*-protected nucleosides (Scheme 2 and Table 1). The known *O*-acetylated derivatives **3a**,**c**,**e**, **5c**, and **7a** were prepared by published procedures,[14] as were the *O*-TBDMS derivatives **3b**,**d**,**f**, **5a**,**b**, and **7b**,**c**.[15] These *O*-protected nucleoside derivatives were ground with $(Boc)_2O$ and DMAP until the reactions were complete as judged by TLC

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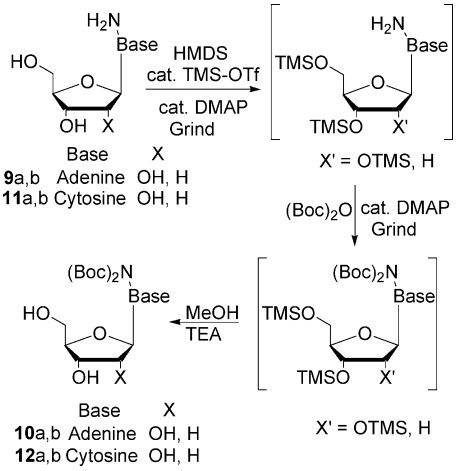
analysis. We observed that as grinding continued the solid reactants formed a liquid phase or melt. The reaction proceeded quickly once the melt was obtained. Model reactions in which grinding was stopped after only a few minutes (to achieve uniform mixing) did not form melts even after standing for several days. TLC analyses of these reactions showed only a small amount of conversion to higher R_f materials. This suggests that grinding the components is necessary, and that even relatively low-energy grinding provides sufficient activation to promote reaction. Grinding may generate "hot spots" leading to localized melting,[10] and the reactants may mix and react in this liquid medium. As the reaction proceeds, melting may continue because of the expected melting point depression of the mixture. In all cases, the mixtures were completely liquid when the reactions were complete. Although one mole of *tert*-butyl alcohol was produced for each mole of $(Boc)_2O$ that reacted, we do not believe that this was sufficient to account for the liquefaction of the reaction mixture.

Although many truly solid-state reactions are known, melt formation during a reaction of initially solid materials is very common, and many reported "solid-state" reactions in fact only proceed in such an intermediate liquid phase. This is true for catalytic transformations (aldol condensations[10b,16] and oligomerizations[17]) and for noncatalytic reactions (Baeyer−Villiger oxidations,[10b,18] oxidative coupling of naphthols,[10b,19] homoetherification of benzylic alcohols,[20] and aromatic bromination[21]).

On completion, the entire mixture was washed from the beads and glassware with a small amount of an appropriate solvent. After concentrating the washings, DMAP was removed from the product by passage through a short silica gel column. As shown in Table 1, acetyl esters, silyl ethers, and acetonide derivatives were unaffected by these conditions. The reactions were complete within 1−7 h, affording a single product in very high yield except in the case of the guanosine derivatives. The lower yields observed for these reactions reflected the formation of several unidentified polar byproducts. Similar modest to low yields are seen in solution-phase reactions of guanosine and its derivatives.[22,23] The bis-*N*-Boc products **4**, **6**, and **8** are new compounds, with the exception of deoxyadenosine derivative **4f**.[7a]

The liquid phase was formed sooner in the reactions of silyl-protected nucleosides, and the reactions were faster and slightly higher yielding than was the case for reactions of ester-protected compounds (Table 1, reactions of **3b** vs **3a**, **3d** vs **3c**, **3f** vs **3e**, **5b** vs **5c**). The faster melt formation in the case of silyl-protected derivatives may reflect the lower melting points of these derivatives compared to acetyl ester derivatives. Our initial observation that the reaction of carboxylic acid **1** formed *tert*-butyl ester **2** (Scheme 1) indicates that carboxyl groups are reactive under these conditions. Presumably **1** formed a mixed anhydride derivative with $(Boc)_2O$ and DMAP, which acylated *tert*-butyl alcohol formed from the breakdown of Boc_2O.

SCHEME 3. Solventless Base Moiety Protection with Transient Silylation of the Sugar Hydroxyls



We next focused on *N*-protecting nucleosides that lacked *O*-protecting groups on their sugar rings. To our knowledge, Boc groups have not previously been installed on the base moiety of nucleosides without prior sugar *O*-protection. Under our solventless conditions, both the sugar hydroxyls and the amino group of the base reacted with $(Boc)_2O$. Limiting the amounts of $(Boc)_2O$ or DMAP catalyst did not give satisfactory results. We realized that in situ transient protection of the hydroxyl groups could be useful to tackle this problem.

Transient protection of the sugar hydroxyls using the trimethylsilyl (TMS) group is standard practice in solution-phase nucleoside chemistry,[23] but it has not been employed in solventless reactions. Adenosine, cytidine, guanosine, and their 2-deoxy analogues were treated with hexamethyldisilazane (HMDS)[24] prior to the introduction of the Boc group. No reaction occurred in the presence of HMDS alone. Adding DMAP did not promote silylation, but adding 3 mole percent of TMSOTf[25] to mixtures already containing HMDS and DMAP led to complete *O*-silylation within 1−3 h. In solution-phase reactions, TMSCl has been employed to promote silylation by HMDS.[24] Although DMAP alone was ineffective as a silylation catalyst, the reactions were very sluggish in its absence. Hence, for the solventless in situ silylation (Scheme 3 and Table 2) the nucleoside was ground with 3−6 equivalents of HMDS along with catalytic amounts of TMSOTf and DMAP until TLC showed complete conversion to product. At this point, all reaction mixtures were liquids. Neat $(Boc)_2O$ was then added, with additional DMAP in some cases. Grinding was continued until the reaction was complete. The complete sequence required no more than 10 h. The *O*-silyl groups were conveniently removed by stirring overnight in methanol containing Et_3N. The bis-*N*-Boc adenosine and cytidine products **10a,b** and **12a,b** were obtained in good yields, as shown in Table 2. In contrast,

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TABLE 2. One Pot Bis-*N*-Boc Protection Using Transient Silylation

	conditions		
substrate	silylation	Boc rxn	product (yield %)
9a	6 equiv HMDS, 3% TMSOTf, 20% DMAP, 3 h	5 equiv $(Boc)_2O$, 10% DMAP, 6−7 h	**10a** (60)
9b	4 equiv HMDS, 3% TMSOTf, 10% DMAP, 3 h	5 equiv $(Boc)_2O$, 20% DMAP, 6−7 h	**10b** (72)
11a	4 equiv HMDS, 3% TMSOTf, 10% DMAP, 2 h	4 equiv $(Boc)_2O$, 4 h	**12a** (65)
11b	3 equiv HMDS, 3% TMSOTf, 10% DMAP, 1 h	4 equiv $(Boc)_2O$, 10% DMAP, 4 h	**12b** (50)

the method afforded complex product mixtures when applied to guanosine or 2-deoxyguanosine.

Conclusions

The solvent-free bis-*N*-Boc protection of the heterocyclic amino group of nucleosides and their derivatives has been demonstrated at 100-milligram through gram scales. These reactions use minimal amounts of reagents and solvents during workup and purification. Transient silylation permits the one-pot solvent free synthesis of bis-*N*-Boc protected nucleosides without *O*-protecting groups on the sugar ring, avoiding tedious multistep sequences. The solventless low-energy ball mill technique is both operationally simpler and more efficient than existing solution-phase methods and thus offers considerable advantages in the preparation of nucleoside derivatives for further synthetic elaboration.

Experimental Section

General Protocol for Solventless Boc Protection. The nucleoside along with $(Boc)_2O$ and DMAP (amounts as stated below) were placed in the ball mill along with 9 mm Pyrex beads. For small-scale reactions (ca. 100−300 mg) 16−20 beads were used; on gram-scale reactions as many as 25−30 were used. The mixture was ground (ca. 120−140 rpm) until the reaction was done as judged by TLC analysis of small aliquots scraped from the reactor and dissolved in a suitable solvent. The thick oily product was washed from the apparatus using several *small* portions of a suitable solvent. The solvent was evaporated and the residue was applied to a short silica gel column, eluting using the solvent(s) indicated. Only 5−10 fractions were required to obtain the product.

***N*^{6},*N*^{6}-bis(*tert*-Butoxycarbonyl)-2′,3′-*O*-isopropylidene-5′-*O*-acetyladenosine (4c).** According to the general procedure, 2′,3′-*O*-isopropylidene-5′-*O*-acetyladenosine **3c** (400 mg, 1.15 mmol), DMAP (14.0 mg, 0.01 mmol), and $(Boc)_2O$ (750 mg, 3.43 mmol) were ground together for 4 h. Chromatography (5% MeOH in CH_2Cl_2) gave **4c** (604 mg, 96%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.38 (s, 3H), 1.43 (s, 18H), 1.61 (s, 3H), 1.93 (s, 3H), 4.19 (dd, J = 5.8, 11.9 Hz, 1H), 4.33 (dd, J = 4.2, 11.9 Hz, 1H), 4.47−4.52 (m, 1H), 5.01 (dd, J = 3.4, 6.2 Hz, 1H), 5.44 (dd, J = 2.1, 6.2 Hz, 1H), 6.16 (d, J = 2.1 Hz, 1H), 8.15 (s, 1H), 8.85 (s, ^{1}H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.5 (CH_3), 25.3 (CH_3), 27.1 (CH_3), 27.7 (6 × CH_3), 63.9 (CH_2), 81.5 (CH), 83.8 (2 × C), 84.2 (CH), 84.9 (CH), 91.2 (CH), 114.7 (C), 129.5 (C), 143.6 (CH), 150.4 (2 × C), 150.6 (C), 152.2 (CH), 152.3 (C), 170.2 (C). $[\alpha]^{25}_D$ = −14.2 (*c* 1.01, CH_2Cl_2). Anal. Calcd for formula $C_{25}H_{35}N_5O_9$: C, 54.64; H, 6.42; N, 12.74. Found: C, 54.32; H, 6.59; N, 12.60.

General Protocol for Transient Silylation and Boc Protection. The nucleoside, hexamethyldisilazane (HMDS), and DMAP were placed in the ball mill apparatus. The indicated amount of TMSOTf was added, and the mixture was ground until TLC showed complete conversion to a higher-running spot. Grinding was halted while $(Boc)_2O$ and additional DMAP (if required) were added. Grinding was then resumed and continued until TLC indicated complete reaction. MeOH plus 20% Et_3N by volume was then added, and the solution was stirred overnight at room temperature to complete the desilylation. The mixture was evaporated, and the residue was applied to a short silica gel column. The product was eluted using the indicated solvent(s).

***N*^{6},*N*^{6}-bis(*tert*-Butoxycarbonyl)adenosine (10a).** Adenosine **9a** (100 mg, 0.374 mmol), HMDS (0.47 mL, 2.24 mmol), DMAP (9.2 mg, 0.07 mmol), and TMSOTf (1.4 μL, 7.5 μmole) were ground together for 3 h. To this was added $(Boc)_2O$ (408 mg, 1.87 mmol) and grinding was continued for a further 6 h. MeOH (25 mL) and TEA (5 mL) were added and stirring was continued overnight. The solvent was evaporated, and the residue was subjected to column chromatography (13% MeOH in CH_2Cl_2), to give **10a** (105 mg, 60%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (s, 18H), 3.71 (dd, J = 1.2, 12.3 Hz, 1H), 3.92 (dd, J = 1.7, 12.3 Hz, 1H), 4.26 (d, J = 1.2 Hz, 1H), 4.34 (dd, J = 1.2, 5.0 Hz, 1H), 4.82 (dd, J = 1.2, 6.6 Hz, 1H), 5.91 (d, J = 6.6 Hz, 1H), 8.21 (s, 1H), 8.81 (s, 1H), broad unresolved signals for 3 hydroxyl protons observed between 3.7 and 5.8 ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 27.7 (6 × CH_3), 62.8 (CH_2), 71.8 (CH), 74.0 (CH), 84.6 (2 × C), 87.3 (CH), 91.1 (CH), 129.8 (C), 144.8 (CH), 150.6 (CH), 150.7 (C), 151.6(2 × C), 152.0 (C). $[\alpha]^{25}_D$ = −45.1 (*c* 1.01, CH_2Cl_2). Anal. Calcd for formula $C_{20}H_{29}N_5O_8$: C, 51.39; H, 6.25; N, 14.98. Found: C, 51.55; H, 6.50; N, 14.83.

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Supporting Information Available: Details of the ball mill apparatus, as well as experimental procedures and spectroscopic data for all reported compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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