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Selective deacylation of peracylated ribonucleosides

Jared W. Rigoli [†], Michael E. Østergaard [†], Kirsten M. Canady, Dale C. Guenther, Patrick J. Hrdlicka [*]

Department of Chemistry, University of Idaho, Moscow, ID 83844-2343, USA

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ABSTRACT

A protocol for chemoselective deprotection of N,O-acylated ribonucleosides has been developed. Peracylated pyrimidine ribonucleosides subjected to guanidinium nitrate and NaOMe in $MeOH/CH_2Cl_2$ at 0 °C undergo high yielding O-deacylation, while even more pronounced chemoselectivity is observed with peracylated purine ribonucleosides as O5′-acyl groups are preserved. Nucleobase-protecting groups (A^{Bz}, C^{Bz}, G^{iBu}, and U^{Bz}) are stable to these conditions, rendering this reagent mixture as a valuable addition to the collection of protecting group protocols in nucleoside chemistry.

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Chemically modified nucleosides and oligonucleotides are widely explored for therapeutic and diagnostic purposes, for example, as antiviral/anticancer agents,[1] as building blocks within the antigene/antisense/siRNA regimes,[2–4] and for detection of single nucleotide polymorphisms.[5] Nucleosides are challenging synthetic substrates as they contain several nucleophilic groups with comparable reactivity (i.e., hydroxyl/amino groups) that must be chemically differentiated for successful multistep synthesis of target nucleosides. Thus, development of methods for chemoselective acylation/deacylation of nucleosides has been a subject of recent interest, and includes direct O-benzoylation of free nucleosides,[6,7] selective N-deacylation of peracylated ribonucleosides,[8] and selective O2′,O3′-deacylation of O2′,O3′,O5′-triacylated ribonucleosides.[9] Methods resulting in selective N-acylation of nucleobase amines/imines are of particular interest since the base moieties of adenosine, cytidine, and guanosine phosphoramidites must be protected for successful automated oligonucleotide synthesis. Selective N-acylation of these moieties has been realized by

(a) Direct chemoselective N-acylation of unprotected nucleosides using mild acylating agents such as aqueous benzoic anhydride.[10] This approach is restricted to cytidine nucleosides, as O-acylation typically accompanies N-acylation with other nucleosides.
(b) Transient protection methodology, that is, a three-step process involving protection of free alcohol groups as silyl ethers, N-acylation and desilylation using a weak base.[11,12]
(c) Peracylation followed by selective O-deacylation. Several reagents have been developed toward this end including (a) sodium methoxide used in the original Khorana approach,[13] (b) aqueous sodium hydroxide in pyridine,[14,15] and (c) dilute methanolic ammonia.[16] Most of these methods furnish the target nucleosides in moderate to good yield.

While exploring an alternative route toward N2′-functionalized 2′-amino-α-L-LNA (Locked Nucleic Acid) monomers,[17] we needed facile access to thymine derivative **1**[17b] (Scheme 1). Chemoselective N3-benzoylation of O2′-unprotected nucleoside **2**[18] using direct N-acylation,[19] phase transfer conditions,[20] or transient protection protocols[11] only resulted in low yields of **1** (<50%) due to formation of O2′-benzoylated and/or O2′,N3-dibenzoylated products.[17b] Alternatively, chemoselective O2′-deacylation of fully protected nucleoside **3**[17b] using the classic O-deacylation methods outlined above (point c) was attempted, but was unsuccessful due to the lability of the N3-benzoyl group and/or the formation of undesired *β-xylo*-LNA nucleosides arising from intramolecular substitution reactions.[17b] Gratifyingly, subjection of **3** to a mixture of guanidinium nitrate (GNO_3) and sodium methoxide in methanol and dichloromethane[21] cleanly afforded O2′-deacylated nucleoside **1** in 96% yield.[17b] This reagent mixture was also found to accomplish O2′-deacetylation of the corresponding 6-*N*-benzoyladenine and O5′/O5″-dibenzoylated thymine derivatives of **1** in similarly high yields.[22] These interesting findings prompted us to evaluate the full potential of this reagent mixture to facilitate selective deacylation of nucleoside substrates.

Peracylated cytosine derivative **4a** was chosen as an easily accessible[23] model compound for qualitative optimization of the deacylation conditions (Table 1). Subjection of **4a** to conditions

* Corresponding author. Tel.: +1 208 885 0108; fax: +1 208 885 6173.
E-mail address: hrdlicka@uidaho.edu (P.J. Hrdlicka).
† To be considered joint first authors.

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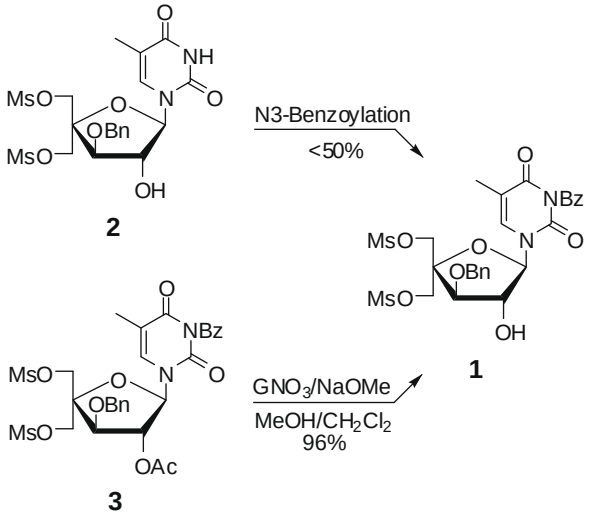
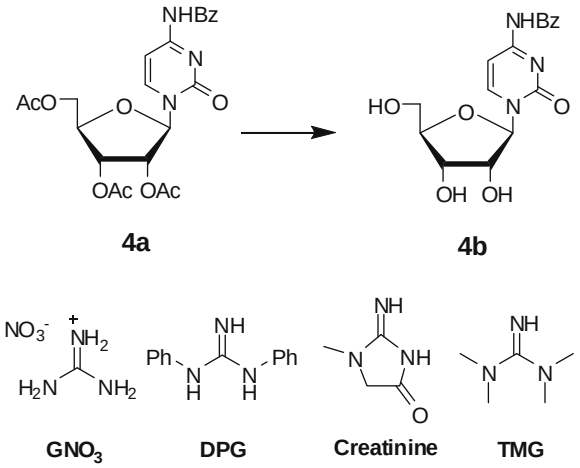
that mimic the original deacylation conditions very closely[17b,21] surprisingly resulted in complete deacylation to exclusively afford cytidine after 1 h (entry 1). Inexpensive analogs of guanidine such as diphenylguanidine (DPG), creatinine, and tetramethylguanidine (TMG) were evaluated as alternative reagents since their altered steric requirements, nucleophilicity and/or basicity may affect the rate and selectivity of the deacylation reactions. Deacylations with DPG and creatinine were slow and non-selective (entries 2 and 3), while the use of TMG resulted in complete deacylation to afford cytidine (entry 4). A decrease in the solvent polarity slowed down the deacylation reaction with TMG and allowed isolation of N4-benzoylcytidine **4b**[23] although only in 40% yield (entry 5). Exchanging the methanol component of the solvent mixture with water, ethanol, *iso*-propanol, acetonitrile, or THF was not advantageous (results not shown). Accordingly, we turned our attention back to the original guanidinium nitrate reagent. If sodium methoxide is excluded from the reagent mixture, the deacylations do not occur (entry 6). Thus, sodium methoxide is necessary to initiate deacylations by GNO_3, most likely by deprotonating the guanidinium to form the active guanidine in situ. Decreasing the relative amount of either guanidinium nitrate or sodium methoxide slowed down deacylations, but did not improve chemoselectivity (entries 7 and 8). Gratifyingly, subjection of peracylated **4a** to the original conditions at lower temperatures (0 °C) successfully resulted in fast and selective O-deacylation and formation of desired 4-*N*-benzoylcytidine **4b**[23] in excellent yield (95%, entry 9).

To investigate the scope of the most promising deacylation condition (Table 1, entry 9), a representative set of fully protected ribonucleosides **5a**–**10a** was selected and synthesized using either established protocols (**5a**,[8] **9a**,[24] and **10a**[25]) or simple protecting group manipulations of readily available nucleosides.[23] The identity of known model nucleosides and deacylated products was verified by comparison of known spectral data (e.g., ^{1}H NMR), while all new compounds reported in this study were characterized by a



Scheme 1. Synthesis of N3-benzoylated nucleoside **1.**[17b]

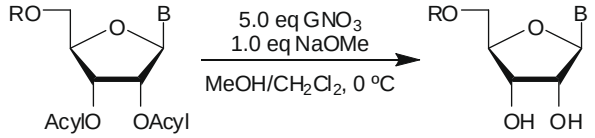
Table 1
Qualitative optimization of reaction conditions for selective O-deacylation of **4a**[a]



Entry	Reagent (equiv)	MeOH:CH_2Cl_2 (v:v)	NaOMe (equiv)	Product	
				1h	**1d**
1	GNO_3 (5.0)	90:10	1.0	C	—
2	DPG (1.0)	50:50	—	Mix	Mix
3	Creatinine (1.0)	90:10	—	NR	Mix
4	TMG (1.0)	90:10	—	C	—
5[b]	TMG (1.0)	50:50	—	4b (40%)[c]	C
6	GNO_3 (5.0)	90:10	—	NR	NR
7	GNO_3 (0.33)	90:10	1.0	Mix	C
8	GNO_3 (5.0)	90:10	0.2	Mix	C
9	**GNO_3 (5.0), 0 °C**	**90:10**	**1.0**	**4b (95%)[c]**	—

[a] Reactions were performed at room temperature using 1.0 equiv of nucleoside **4a** (0.02 M) unless otherwise noted. C = cytidine, Mix = formation of complex mixture with multiple products, NR = no reaction.
[b] reaction performed using a 0.1 M solution of **4a**. Nucleoside **4b** was formed after 20 min.
[c] Isolated yield.

Table 2
Deacylation of model nucleosides using guanidinium nitrate (GNO_3) and sodium methoxide[a]



4a-10a **4b-10b**

Substrate				Product			Time (min)	Yield (%)
	R	Acyl	B		R	B		
4a	Ac	Ac	C^{Bz}	**4b**	H	C^{Bz}	90	95
5a	Ac	Ac	C^{Ac}	**5b**	H	C	10	95
6a	DMTr	Ac	U^{Bz}	**6b**	DMTr	U^{Bz}	30	85
7a	Ac	Ac	$A^{Ac,Bz}$	**7b**	Ac	A^{Bz}	15	60
8a	Ac	Ac	G^{iBu}	**8b**	Ac	G^{iBu}	180	80
9a	Bz	Bz	U	**9b**	Bz	U	24 h	20
10a	Bz	Bz	A^{Bz}	**10b**	Bz	A^{Bz}	20	75

[a] Reactions were performed as described for entry 9 in Table 1. C^{Bz} = 4-*N*-benzoyl-cytosin-1-yl, C^{Ac} = 4-*N*-acetyl-cytosin-1-yl, C = cytosin-1-yl, G^{iBu} = 2-*N*-isobutyryl-guanin-9-yl, $A^{Ac,Bz}$ = 6-*N*-acetyl,6-*N*-benzoyl-adenin-9-yl, A^{Bz} = 6-*N*-benzoyl-adenin-9-yl, U^{Bz} = 3-*N*-benzoyl-uracil-1-yl, DMT = 4,4′-dimethoxytrityl.

combination of ^{1}H NMR (including D_2O exchange studies), COSY, or FAB-MS. The selected model nucleosides **4a**–**10a** feature alcohol-protecting groups that are commonly encountered in nucleoside chemistry (–OAc, –OBz, ODMTr). The nucleobase moieties were protected with acyl groups that are commonly used in standard solid phase oligonucleotide synthesis (A^{Bz}, C^{Bz}, C^{Ac}, G^{iBu}).

The O2′/O3′-acyl groups (OAc/OBz) of all studied nucleosides were cleaved using the optimized conditions, while most nucleobase-protecting groups, including A^{Bz}, C^{Bz}, G^{iBu}, and the challenging U^{Bz}, were stable to these conditions (Table 2). Only the highly labile *N*-acetyl groups of cytidine and adenosine derivatives **5a** and **7a**, respectively, were cleaved under these conditions. Interestingly, O5′-acyl groups (OAc/OBz) of purines were left unharmed (**7a**, **8a**, and **10a**), while they were cleaved in pyrimidine substrates (**4a** and **5a**). It was possible, however, to isolate O5′-benzoylated uridine derivative **9b** albeit in very low yield. The commonly used DMTr-protecting group was fully stable to these deacylation conditions. Thus, selective O-deacylation of suitably protected pyrimidines proceeds in excellent yields (85–95%, Table 2), while selective O2′,O3′-deacylation of peracylated purines proceeded in good yield (60–80%, Table 2).

To sum up, the guanidinium nitrate/sodium methoxide reagent mixture presented herein facilitates selective *O*-deacylation of ribonucleosides with N-acylated nucleobase moieties (A^{Bz}, C^{Bz}, G^{iBu}, T^{Bz}, and U^{Bz}). A special selectivity is observed with peracylated purines as O2′,O3′-deacylated products are exclusively formed. The examples presented herein (Scheme 1 and Table 2) underline that the guanidinium nitrate/sodium methoxide reagent mixture is a valuable addition to the collection of protecting group protocols in nucleoside chemistry.

Representative deacylation protocol: A stock solution of guanidinium nitrate (1.24 g, 10 mmol) and sodium methoxide (108 mg, 2.0 mmol) in MeOH (90 mL) and CH_2Cl_2 (10 mL) was prepared. Protected nucleoside (0.1 mmol) was dissolved in 5 mL of this solution at 0 °C. Upon completion of the reaction, 5% aq. citric acid was added and the aqueous phase was extracted with CH_2Cl_2. After evaporation of solvents, the resulting crude was purified by silica gel chromatography using methanol in CH_2Cl_2 as eluent.

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Supplementary data

Experimental description and characterization data of compounds **4a**–**10a** and **4b**–**10b** are available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.01.147.

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