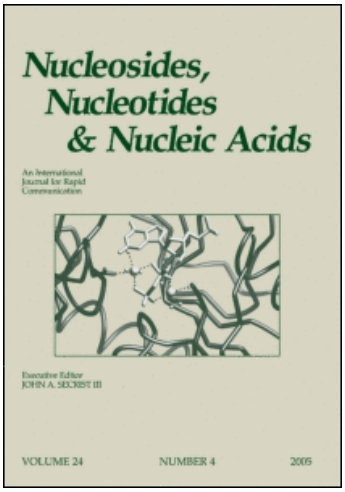

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BORANE-AMINE COMPLEXES - VERSATILE REAGENTS IN THE CHEMISTRY OF NUCLEIC ACIDS AND THEIR ANALOGS

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ABSTRACT

A new method for synthesis of N-alkylated nucleosides was developed. Exceptionally mild and selective conversion of N-acyl to the corresponding N-alkyl nucleosides was achieved by reduction with borane-amine complexes. The borane-amine complexes were also used as efficient scavengers of a 4,4′-dimethoxytrityl (DMT) cation. Neutralization of the cation eliminated the boranophosphate group degradation during acidic DMT deprotection and allowed milder acidic conditions for the deprotection.

INTRODUCTION

Borane-amine complexes (BH_3-amine) are commercially available reagents widely used in organic synthesis for hydroboration and selective reductions, and as stabilizers and polymerization catalysts (1). They also find applications in nucleic acids chemistry. Most commonly, borane-amine complexes have been used as borane carriers for exchange reactions (2–6). Borane exchange with phosphite triesters proceeds smoothly generating boranophosphate triesters in near quantitative yield. It was successfully exploited in the synthesis of oligonucleoside boranophosphates (BH_3^--ODN) by phosphoramidite (2) and H-phosphonate (3) methods, nucleoside (α-borano)triphosphates (4), new oligonucleoside boranophosphorothioate analogs (5), and several carbohydrate boranophosphate analogs (6). Development of the boranophosphate nucleic acids chemistry brings to light other useful applications

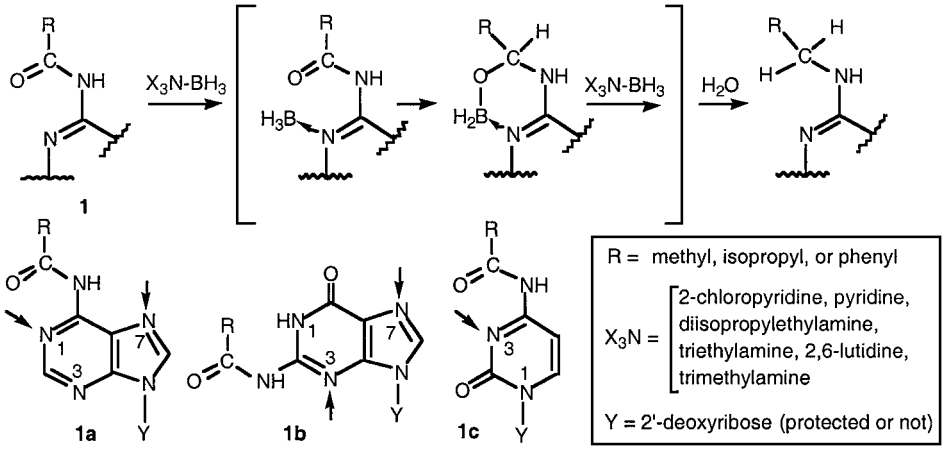
of the borane-amine complexes. Here we report their utility as mild and selective reducing agents and as carbocation scavengers.

RESULTS AND DISCUSSION

Synthesis of N-alkylated nucleosides by reduction with borane-amine complexes. Borane-amine complexes reacting with nucleic acids can exchange the borane moiety not only with phosphite triesters but also with heterocyclic base nitrogens having a lone electron pair. Such complexes are unstable and the borane dissociates upon treatment with a base such as hydrazine or ammonia. Being reversible and harmless for the plain nucleosides, the borane-nucleobase complex formation is suggested to be involved in a surprisingly facile reduction of an N-acyl group in the base-protected nucleosides (7) via a mechanism proposed in Scheme 1.

The reduction of several common acyl-protecting groups rendered the corresponding N-alkyl derivatives in good yields (Table 1). Amides are usually inert toward borane-amine complexes; their reduction to the corresponding amines requires THF-borane complex at elevated temperatures (8). However, formation of the borane complex with a nucleobase nitrogen may juxtapose the amide and borane groups as in Scheme 1, thereby diminishing the activation energy barrier. Additionally, borane coordination (**1a–c**) will decrease to some extent the electron density on heterocyclic rings and increase the positive charge on the amide carbon, facilitating the reduction. The combination of these factors could explain the unusual susceptibility of the amide group in the nucleosides toward reduction by relatively inactive borane-amine complexes (7).

Exceptionally mild conditions used for the exocyclic amide group reduction create the basis for the selective reactions. Selectivity of the reduction was



Scheme 1. Proposed mechanism of the nucleoside N-acyl protecting group reduction. Arrows indicate possible sites of borane coordination in the acyl protected nucleosides.

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Table 1. Nucleosides Tested in the Reaction (Scheme 1), Reaction Times and Yields[a]

Starting Nucleoside	Product	Reaction Time	Yield, %
N^6-benzoyl-2′-dA	N^6-benzyl-2′-dA	20 min	85
N^4-benzoyl-2′-dC	N^4-benzyl-2′-dC	1 h	97
N^2-isobutyryl-2′-dG	N^2-isobutyl-2′-dG	10 h	73
3′-O,N^4-dibenzoyl-2′-dC	3′-O-benzoyl-N^4-benzyl-2′-dC	2 h	74

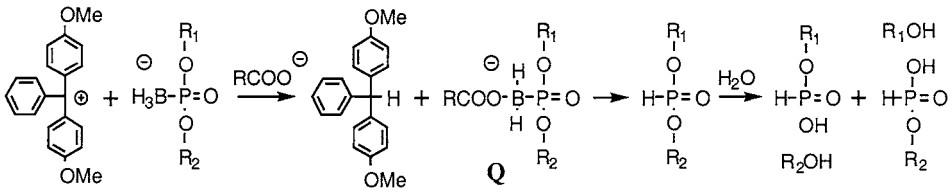
[a]Reactions were carried out in THF with 0.05 M nucleoside, 0.25 M BSA, and 0.5 M BH_3-DIPEA at 25°C.

demonstrated on nucleosides having both base- and deoxyribose-protecting groups. As was expected, the reduction of only N-acyl base-protecting groups was observed while the deoxyribose protecting groups remained intact (7).

Use of borane-amine complexes as scavengers for DMT cations. 4,4′-Dimethoxytrityl (DMT) is the most widely used hydroxyl protecting group in nucleic acids chemistry. Although convenient, it suffers certain disadvantages. The presence of highly stabilized DMT^+ cations in the reaction mixture during deblocking not only makes the reaction reversible and decreases overall yield but also could lead to undesirable reactions with some functional groups such as BH_3 or SH.

As part of our on-going investigations, we are interested in the synthesis of oligonucleoside boranophosphates. We and others observed substantial decomposition of a boranophosphate group during acidic DMT deprotection (3). A possible mechanism of the reaction of borane group with DMT^+ is outlined on Scheme 2. The carbocation abstracts hydride from the borane group forming dimethoxytritan, and the coordinatively unsaturated boron intermediate reacts with the acid anion to give adduct **Q**. Further decomposition of compound **Q** via intermediates of unknown structure generates H-phosphonate diester. Finally, the H-phosphonate hydrolysis results in the internucleoside bond cleavage.

Borane-amine complexes are known readily react with trityl cations yielding tritans (9). We suggested that borane amine complexes, being in excess in the reaction mixture during DMT hydrolysis, could serve as scavengers and "protect" nucleoside boranophosphates from the decomposition. For our studies we used protected dithymidine boranophosphates with three types of boranophosphate linkage (Table 2, compounds I–III) both in solution and on solid phase. As potential scavengers we tested borane-pyridine and borane-trimethylamine complexes as well



Scheme 2. Degradation of the boranophosphate bond during acidic DMT deprotection.

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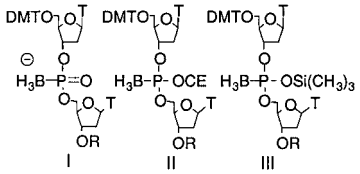
Table 2. Yields of the Dithymidine Boranophosphate After DMT Deprotection of Dimers I–III[a]



Scavenger (0.1 M)	In Solution		On Solid Support		
	I	II	I	II	III
No scavenger	19	80	21	88	38
BH_3-Py	90	94	89	89	79
BH_3-Me_3N	60	–	55	–	–
Et_3SiH	–	–	15	56	17

[a]DMT-removal reaction was performed in a 2% solution of dichloroacetic acid in dichloromethane for 5 min with or without the presence of one of the scavengers. The reaction in solution was stopped by TEA addition (1 eq. to the acid), the solution was evaporated and treated with ammonia for 1 h; the polymer support after DMT removal was washed with dichloromethane, treated with ammonia for 1 h and analyzed by RP HPLC.

as triethylsilane, a carbocation scavenger, commonly used in peptide and nucleic acids chemistry.

The reaction mixtures after DMT deprotection were analyzed by RP HPLC. Along with dithymidine boranophosphate and cleavage products, thymidine and thymidin-3′/5′- yl H-phosphonates, we were able to isolate the adduct **Q**, containing a dichloroacetate moiety. This compound appears to be sufficiently stable to survive HPLC. The ^{1}H, ^{31}P NMR and MS spectra of the purified product **Q** are consistent with the proposed structure.

The results indicate that the stability of the boranophosphate group during acidic DMT removal depends on the presence and the nature of the protection group at phosphorus. While boranophosphate diester I underwent significant degradation, dimer II with β-cyanoethyl protection was relatively stable in these conditions, especially on solid phase. Dimer III, having a labile silyl protecting group, appeared to be less stable than dimer II. Addition of borane-amine scavengers indeed significantly suppressed boranophosphate group degradation during acidic DMT removal for all dimers used. Borane-pyridine complex was the most effective, almost completely eliminating the degradation (Table 2). Yet the commonly used carbocation scavenger, triethylsilane, was not effective in these conditions, causing substantive damage of the boranophosphate bond.

It is noteworthy that use of a borane-amine scavenger allows for milder and faster DMT group deprotection. Thus, the deprotection of 5′-DMT-thymidine in 2% monochloroacetic acid was complete in 30 min in the presence of 0.1 M borane-pyridine complex, whereas it was incomplete after 1 h in the absence of the scavenger.

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