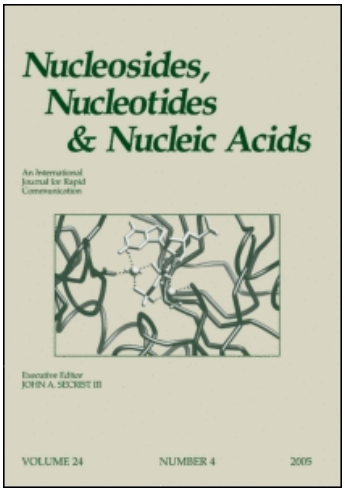

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Rapid and Selective Reduction of Amide Group by Borane-Amine Complexes in Acyl Protected Nucleosides

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RAPID AND SELECTIVE REDUCTION OF AMIDE GROUP BY BORANE-AMINE COMPLEXES IN ACYL PROTECTED NUCLEOSIDES.

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Dedicated to the memory of Dr. Gertrude B. Elion

ABSTRACT. Borane-amine complexes provide an unusually fast and selective reduction of a deoxynucleoside N-acyl group to a corresponding N-alkyl group. Three different nucleosides (dG, dA, and dC) each having one of three N-protecting groups (benzoyl, isobutyryl, or acetyl) were used to prepare N-alkylated nucleosides in good yields under mild conditions. Deoxyribose O-acyl protecting groups remain intact at the conditions of N-acyl group reduction.

INTRODUCTION

During the past decade, nucleoside and nucleotide analogs steadily gained importance as anti-viral and anti-neoplastic agents.[1-6] The efficacy of several modified nucleosides against difficult to cure diseases, such as cancer and AIDS, prompted researchers to search for other analogs and to develop efficient methods for their synthesis.[5-7] In addition to the existing arsenal of chemical methods aimed at the synthesis of modified nucleosides, we report here a new procedure for the synthesis of nucleoside derivatives alkylated at an exocyclic amino group via mild reduction of an N-acyl protecting group with borane complexes.

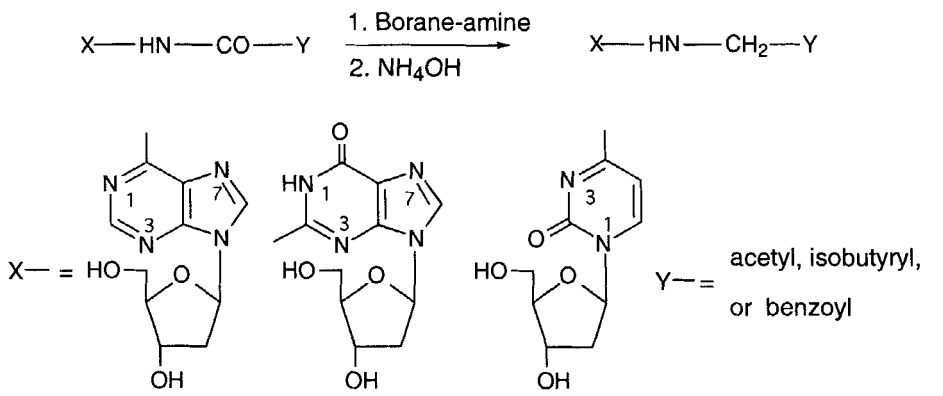
Borane is widely used as a reducing agent in organic syntheses.[8,9] Diborane and different borohydrides can reduce nearly any functional group in an organic molecule including relatively inert ether, nitrile, or amide groups.[9-11] Borane complexes with Lewis bases, such as sulfides or amines, are milder and more selective reducing agents.[12] Borane-alkylamines reduce aldehydes, ketones, and acyl chlorides, whereas carboxylate anions, esters, and amides are resistant to these agents.[12,13] However, the reactivity of a given functional group can be substantially modified by the organic structure to which it is attached. In the present article we describe the use of a number of borane-amine complexes

to provide an unusually expeditious reduction of the amide functional group under mild conditions in protected nucleosides.

RESULTS AND DISCUSSION

We studied several commercially available nucleosides containing different N-acyl protecting groups (SCHEME 1). The compounds were first solubilized in tetrahydrofuran (THF) by silylation with N,O-bis(trimethylsilyl)acetamide (BSA) and then reacted with borane-N,N-diisopropyl-N-ethylamine complex (BH_3-DIPEA) at ambient temperature. The reaction afforded a mild reduction of an exocyclic N-acyl protecting group to a corresponding alkyl group. The reduction of all tested acyl groups proceeded smoothly and was conveniently followed by TLC, ^{1}H NMR, and RP HPLC. The rate of the amide-group reduction varied for different nucleosides. Acyl groups at the N^6 position of deoxyadenosine were reduced most easily; the benzoyl group, for example, required only 20 min for complete reaction. The N^4-acyl groups of deoxycytidine were less reactive, requiring 1-3 h for complete reduction. The least reactive compounds were N^2-acyl deoxyguanosine derivatives. Among the studied acyl protecting groups, benzoyl was the most labile to reduction by borane-amine (TABLE 1).

Scheme 1



During reduction of the acyl groups, a borane exchange reaction between borane-amine and nucleophilic centers of nucleobases (N3 of cytosine, N7 of guanine, and N1, N7 of adenine) can occur to some extent, as was shown in our earlier studies.[14-16] The complexes formed are weak, however, and are readily reversed in the presence of a competitor base.[14] To reverse formation of any such borane-base complexes, the reaction mixtures were therefore treated at conditions regularly used to deprotect oligonucleotides, e.g., concentrated ammonia for 12 h at 55 °C (conditions were not optimized). The newly formed alkylamine linkage is stable at these conditions, and N-alkylated nucleosides were isolated with high yields by RP HPLC (TABLE 1).

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^6-isobutyryl-2'-dA	N^6-isobutyl-2'-dA	1 h	65
N^4-benzoyl-2'-dC	N^4-benzyl-2'-dC	1 h	97
N^4-isobutyryl-2'-dC	N^4-isobutyl-2'-dC	2 h	82
N^4-acetyl-2'-dC	N^4-ethyl-2'-dC	3 h	83
N^2-benzoyl-2'-dG	N^2-benzyl-2'-dG	4 h	74
N^2-isobutyryl-2'-dG	N^2-isobutyl-2'-dG	10 h	73

[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.

TABLE 2. Reduction of N^6-benzoyl-2'-deoxyadenosine by borane-amine complexes in order of decreasing activity.[a]

Borane-amine complex	Reaction time	Yield, %
Borane-2-chloropyridine	5 min	82
Borane-diisopropylethylamine	20 min	85
Borane-triethylamine	4.5 h	59
Borane-lutidine	8 h	74
Borane-pyridine	8 h	18
Borane-trimethylamine	6 h[b]	27

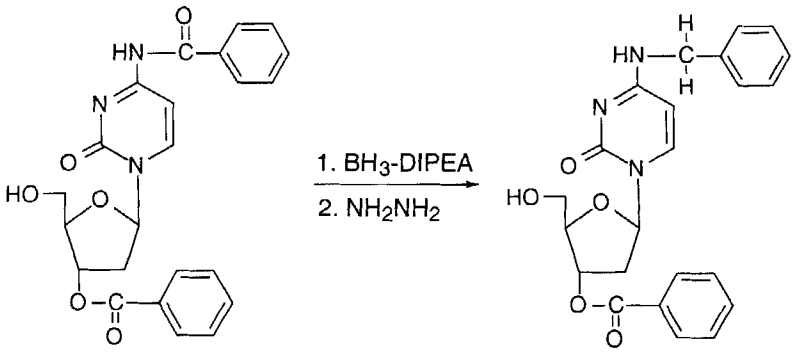
[a]The reaction conditions were the same as in TABLE 1. Yields were not optimized.
[b]At 50 °C.

Six borane-amine complexes were studied as reducing agents for the reaction presented in SCHEME 1. All complexes converted benzoyl to the corresponding benzyl group in N^6-protected deoxyadenosine. However, the reduction rates were quite different (TABLE 2). As was expected, the reactivities of the borane-amine complexes strongly correlated with their relative stability. A similar correlation was also reported by others for reductive

reactions with borane-amines.[12,17] The least reactive borane-trimethylamine complex required an elevated temperature to achieve measurable reduction of the acyl group; the reaction was incomplete after 6 h.

An important feature of the studied reaction is its selectivity. As mentioned, the borane-amine complexes are usually inert toward amides and esters. To demonstrate the selectivity of the reduction, we studied nucleosides that were protected at both the heterocyclic base and deoxyribose with benzoyl groups (SCHEME 2). The reduction of 3'-O, N^4-dibenzoyl-2'-deoxycytidine with BH_3-DIPEA complex was performed at the same conditions as in TABLE 1 (2 h at 25 °C) and was followed by mild treatment with hydrazine to reverse any base boronation. As was expected, the borane-amine reduced only the N-benzoyl protecting group at the heterocyclic base; the O-benzoyl protecting group on deoxyribose remained unchanged. The product, 3'-O-benzoyl-N^4-benzyl-2'-deoxy-cytidine, was isolated in good yield (74%) by RP HPLC and characterized by UV, ^1H NMR spectroscopes and FAB mass spectrometry. We likewise observed selective reduction of base-protecting acyl groups in 3'-O, N^6-dibenzoyl-2'-deoxyadenosine and 3'-O-benzoyl, N^2-isobutyryl-2'-deoxyguanosine, leaving intact the deoxyribose protecting acyl group.

Scheme 2



The facile reduction of the amide group in protected nucleosides might be explained by formation of an intermediate complex between the borane and the heterocyclic base.[14-16] Proposed coordination of the borane to N3 of cytosine, N7 of guanine, and N1 or N7 of adenine could promote the reduction in two ways. First, the complex formation juxtaposes the borane moiety and the amide group, making feasible an intramolecular transfer of a borane hydrogen to the amide carbon. Second, formation of the donor-acceptor N–B bond diminishes the electron density on the heterocyclic ring and exocyclic amide group, facilitating the reduction of the amide carbon.

Thus, the observed differences in reduction rates among the nucleosides could result from differences in the sites and affinities of borane-base complexes. The position of the

BH_3-moiety in N1- and N7-deoxyadenosine-borane complexes favors reduction of the nearby N^6-amide group. Likewise, the boronation at the N3 position on deoxycytidine favors an N^4-amide group reduction. In contrast, the N^2 position of deoxyguanosine is relatively far from N7, which is the preferred site on the heterocycle for borane complex formation. The formation of a coordination complex with N3 of the guanine ring would bring the borane moiety close to the N^2-amide group. However, we did not observe formation of a complex at N3 guanosine in our earlier studies with cyanoborane,[14-16] and we therefore infer that the analogous complex with BH_3, if formed, would be unstable. Instability of this complex would hinder the efficient reduction of the amide group in deoxyguanosine, explaining the experimental data (TABLE 1).

Indirect evidence of the important role of the heterocyclic base nitrogens in amide reduction was demonstrated in a model experiment. Benzanilide, a close analog of an acyl-protected nucleobase, was incubated with BH_3-DIPEA complex for 2 h at 25 °C, e.g., at the conditions used for the complete reduction of N^4-benzoyl-2'-deoxycytidine. Post reaction analysis revealed that practically no reduction (<2%) of the amide group occurred in this analog, which is lacking ring nitrogens.

In conclusion, an efficient method for the synthesis of nucleoside derivatives alkylated at an exocyclic amino group has been developed. The method uses mild reductive conditions and is highly selective toward exocyclic amide groups of nucleobases. The study also provides important information about the mechanism of interaction of borane and its complexes with nucleobases, nucleosides, and nucleotides.

EXPERIMENTAL

Materials and Methods: N,O-bis(trimethylsilyl)acetamide (BSA), borane-N,N-diisopropylethylamine (BH_3-DIPEA), borane-pyridine, borane-trimethylamine, borane-triethylamine, borane-lutidine were purchased from Aldrich Chemical Co. Borane-2-chloropyridine complex was prepared as described.[18] Tetrahydrofuran (Fisher Scientific) was dried before use by distillation from sodium-benzophenone. N^4-Isobutyryl-2'-deoxycytidine, N^4-benzoyl-2'-deoxycytidine, N^6-benzoyl-2'-deoxyadenosine and N^2-isobutyryl-2'-deoxyguanosine were purchased from Chem-Impex International. N^4-Acetyl-2'-deoxycytidine was purchased from ChemGenes; 3'-O-, N^4-dibenzoyl-2'-deoxycytidine, 3'-O-, N^6-dibenzoyl-2'-deoxyadenosine, and 3'-O-benzoyl-N^2-isobutyryl-2'-deoxyguanosine were purchased from Sigma; 5'-dimethoxytrithyl-N^6-isobutyryl-2'-deoxyadenosine was a gift from Monomer Sciences Inc. N^2-Benzoyl-2'-deoxyguanosine was synthesized as described.[19]

High-performance liquid chromatography was performed on a Waters 600E controller system equipped with a 991 photodiode array detector. Reversed-phase

chromatography was carried out on a 7.8x300 mm Delta Pak C18 (15 mm) column. Samples were eluted with linear gradient 0-40% acetonitrile in 0.02 M triethylammonium bicarbonate (TEAB, pH 7.7).

^{1}H NMR spectra were recorded on a Varian Inova 400 spectrometer at 399.9 MHz frequency. Fast atom bombardment mass spectrometry (FAB-MS) data were obtained on a JEOL-300 mass spectrometer in positive ion mode. UV spectra were recorded on a Milton Roy Spectronic 3000 Array spectrometer.

General procedure for the reduction (SCHEME 1). N-acyl nucleoside (0.05 mmol) was dried over P_2O_5 and suspended in dry THF (1 ml). BSA (62 ml, 0.25 mmol) was added and after the solution became clear (10-20 min) BH_3-DIPEA (88 ml, 0.5 mmol) was added to the reaction mixture. After stirring at room temperature for appropriate periods of time (5-120 min), the reaction mixture was diluted with 5 ml MeOH and evaporated to dryness. Concentrated ammonia (10 ml) was added and the mixture was incubated at 55 °C for 12 h. After evaporation the mixture was dissolved in 0.02 M TEAB and applied on RP HPLC.

N^6-benzyl-2'-deoxyadenosine. Yield - 85%. l_{max} (MeOH) 270 nm. ^{1}H NMR (DMSO-d_6): d 8.42 (s, 1H, 8-H); 8.12 (s, 1H, 2-H); 7.20 (m, 5H, Bn); 6.28 (dd, 1H, 1'-H); 4.80 (br.s, 2H, CH_2); 4.62 (m, 1H, 5'-OH); 4.42 (m 1H, 3'-OH); 4.37 (m, 1H, 3'-H); 3.82 (m, 1H, 4'-H); 3.59 (m, 2H, 5', 5"-H); 2.64 (m, 1H, 2'-H); 2.20 (m, 1H, 2'-H). FAB-MS m/z 343.47 (MH^+, calcd 343.27).

N^6-isobutyl-2'-deoxyadenosine. Yield - 65%. l_{max} (MeOH) 268 nm. ^{1}H NMR (D_2O): d 8.04 (s, 1H, 8-H); 7.97 (s, 1H, 2-H); 6.25 (t, 1H, 1'-H); 4.52 (m, 1H, 3'-H); 4.08 (m, 1H, 4'-H); 3.65 (m, 2H, 5', 5"-H); 3.15 (m, 2H, CH_2); 2.66 (m, 1H, 2'-H); 2.44 (m, 1H, 2'-H); 1.78 (m, 1H, CH); 0.79 (s, 3H, CH_3), 0.77 (s, 3H, CH_3). FAB-MS m/z 308.14 (MH^+, calcd 308.35).

N^4-benzyl-2'-deoxycytidine. Yield - 97%. UV: l_{max} (MeOH) 243, 274 nm. ^{1}H NMR (D_2O): d 7.55 (d, 1H, J = 7.6 Hz, H-6); 7.20 (m, 5H, Bn); 6.09 (t, 1H, 1'-H); 5.84 (1H, d, J = 7.6 Hz, H-5); 4.40 (s, 2H, CH_2); 4.24 (m, 1H, 3'-H); 3.86 (m, 1H, 4'-H); 3.62 (m, 2H, 5', 5"-H); 2.21 (m, 1H, 2'-H); 2.12 (m, 1H, 2'-H). FAB-MS m/z 318.15 (MH^+, calcd 318.35).

N^4-isobutyl-2'-deoxycytidine. Yield - 82%. UV: l_{max} (MeOH) 246, 273 nm. ^{1}H NMR (D_2O): d 7.49 (d, 1H, J = 7.6 Hz, H-6); 6.10 (t, 1H, 1'-H); 5.80 (1H, d, J = 7.6 Hz, H-5); 4.25 (m, 1H, 3'-H); 3.86 (m, 1H, 4'-H); 3.62 (m, 2H, 5', 5"-H); 3.00 (d, 2H, CH_2); 2.24 (m, 1H, 2'-H); 2.12 (m, 1H, 2'-H); 1.70 (m, 1H, CH); 0.75 (s, 1H, CH_3); 0.74 (s, 1H, CH_3). FAB-MS m/z 284.24 (MH^+, calcd 284.32).

N^4-Ethyl-2'-deoxycytidine. Yield - 83%. UV: λ_{max} (MeOH) 239, 273 nm. ^1H NMR (DMSO-d6): d 7.67 (d, 1H, J = 7.6 Hz, H-6); 6.10 (dd, 1H, 1'-H); 5.66 (1H, d, J = 7.6 Hz, H-5); 5.12 (m, 1H, 5'-OH); 4.88 (m 1H, 3'-OH); 4.13 (m, 1H, 3'-H); 3.69 (m, 1H, 4'-H); 3.47 (m, 2H, 5', 5''-H); 3.18 (m, 2H, CH_2); 2.03 (m, 1H, 2'-H); 1.85 (m, 1H, 2'-H); 1.03 (m, 3H, CH_3). FAB-MS m/z 256.02 (MH^+, calcd 256.27).

N^2-benzyl-2'-deoxyguanosine. Yield - 74%. λ_{max} (MeOH) 258 nm. ^1H NMR (DMSO-d6): d 7.85 (s, 1H, H-8); 7.32-7.20 (m, 5H, Bn); 6.08 (t, 1H, 1'-H); 5.20 (m, 1H, 5'-OH); 4.81 (m 1H, 3'-OH); 4.44 (s, 2H, CH_2); 4.27 (m, 1H, 3'-H); 3.71 (m, 1H, 4'-H); 3.46 (m, 2H, 5', 5''-H); 2.53 (m, 1H, 2'-H); 2.10 (m, 1H, 2'-H). FAB-MS m/z 358.02 (MH^+, calcd 358.35).

N^2-isobutyl-2'-deoxyguanosine. Yield - 73%. λ_{max} (MeOH) 256 nm. ^1H NMR (D_2O): d 8.10 (s, 1H, H-8); ; 6.48 (t, 1H, 1'-H); 4.79 (m, 1H, 3'-H); 4.23 (m, 1H, 4'-H); 3.94 (m, 2H, 5', 5''-H); 3.42 (d, 2H, CH_2); 3.04 (m, 1H, CH); 2.61 (m, 1H, 2'-H); 2.13 (m, 1H, 2'-H); 1.17 (s, 3H, CH_3); 1.16 (s, 3H, CH_3). FAB-MS m/z 324.20 (MH^+, calcd 324.66).

3'-O-benzoyl-N^6-benzyl-2'-deoxycytidine. Starting 3'-O-, N^6-dibenzoyl-2'-deoxy-cytidine (10.8 mg, 0.025 mmol) was dried over P_2O_5 and suspended in dry THF (1 ml). BSA (31 ml, 0.125 mmol) was added followed in 20 min by BH_3-DIPEA (44 ml, 0.25 mmol). After 2h of stirring at room temperature, the reaction mixture was diluted with MeOH and evaporated to dryness. The residue was dissolved in Py:AcOH (4:1) buffer and treated with hydrazine-hydrate (25 ml) for 15 min. Reaction was quenched by acetylacetone (200 ml) and the mixture was evaporated to dryness and coevaporated with toluene. The residue was dissolved in H_2O/CH_3CN (1:1) mixture and applied on RP HPLC. (Delta Pak C18 column; 20% CH_3CN in 0.02 M TEAB) .
Yield - 74%. λ_{max} (MeOH) 274 nm. ^1H NMR (DMSO-d6): d 8.18 (t, 1H, NH); 7.97 (m, 2H, Bz); 7.82 (d, 1H, H-6, *J*=7.6 Hz); 7.66 (m, 1H, Bz); 7.51 (m, 2H, Bz); 7.27 (m, 5H, Bn); 6.26 (dd, 1H, 1'-H); 5.84 (d, 1H, H-5, *J*=7.6 Hz); 5.43 (m, 1H, 3'-H); 5.16 (br.s, 1H, 5'-OH); 4.45 (d, 2H, CH_2); 4.12 (m, 1H, 4'-H); 3.68 (m, 2H, 5', 5''-H); 2.38 (m, 1H, 2'-H); 2.24 (m, 4H, 2'-H). FAB-MS m/z 422.21 (MH^+, calcd 422.45).

Comparison of reducing properties of different borane-amine complexes. The study was performed with N^6-benzoyl-2'-deoxyadenosine as model compound (17.8 mg, 0.05 mmol) and 10 equivalents (0.5 mmol) of a borane-amine complex in dry THF (1 ml). Six complexes listed in TABLE 2 were tested as reductants. The reaction was performed at room temperature, except that the borane-trimethylamine complex reacted at 50 °C. After appropriate periods of time aliquots were withdrawn and treated with concentrated ammonia at 55 °C for 12 h. After evaporation the residues were dissolved in water and analyzed by RP HPLC (Delta Pak C18 column; 0-40% CH_3CN in 0.02 M TEAB).

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