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The Use of Aluminum Chloride for Removal of Boc Groups on 5'-Amino-2', 5'-Dideoxyoligonucleotides During Solid-Phase Synthesis

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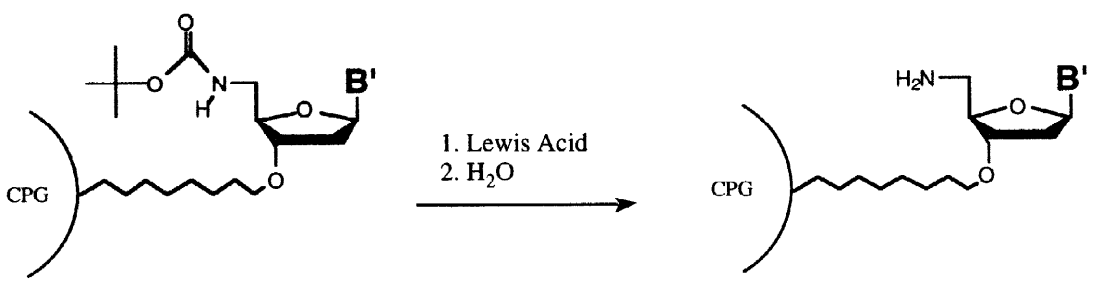
Abstract: *A method for the facile removal of the t-butoxycarbonyl (Boc) group with aluminum chloride during solid-phase oligonucleotide synthesis is presented. The method enables the common amino protecting group, which is stable to base and weak acid, to be removed with no threat to the integrity of the oligonucleotide. The suitability of several other Lewis acids for this procedure is discussed.* © 1997 Elsevier Science Ltd. All rights reserved.

Recent years have seen considerable progress in the use of solid-phase methodologies for generating chemical libraries of small, organic molecules as well as for synthesizing biopolymers such as oligonucleotides and polypeptides.[1] With the demand for chemical libraries of increased diversity ever increasing, so the need for adapting solution-phase chemistry to solid-phase chemistry increases. Consequently, a wider variety of reagents that are compatible with solid-phase synthesis need to be at the chemists' disposal.

We recently reported efficient syntheses of *N*-Boc-5'-amino-2', 5'-dideoxynucleoside-3'-O-phosphoramidites and their incorporation into oligonucleotides via solid-phase synthesis in order to obtain substrate libraries for *in vitro* selection experiments.[2] In that work, dilute mixtures of trifluoroacetic acid in methylene chloride were used for the removal of the *t*-butoxycarbonyl (Boc) group. Although no depurination was observed for purines that were internal in resin-bound oligonucleotides, purines at the 5' terminus were vulnerable and some loss due to exposure to the acid treatment was evident. We therefore tested a variety of aprotic acids to find conditions sufficiently selective to remove the Boc group quantitatively during solid-phase synthesis without resulting in depurination (Scheme 1).



Scheme 1. Oxyphilic, aprotic acids were tested for the ability to remove the *t*-butoxycarbonyl (Boc) group during solid-phase oligonucleotide synthesis. A suitable reagent had to be efficient for comprehensive removal of Boc, compatible with the DNA, and accommodating to solid-phase techniques.

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Several methods for removing Boc groups from amines have been reported.[3-5] Silicon-based reagents such as trimethylsilyl iodide[3] and trimethylsilyl bromide were both found to remove the Boc group quite effectively. However, depurination occurred at the concentrations necessary for deprotection. Bromocatecholborane[5] also removed the Boc group rather efficiently, but unfortunately adenosine was unstable to this reagent.[6]

The most suitable reagent that we tested was aluminum chloride ($AlCl_3$). During studies of β-lactam antibiotics, Tsuji and coworkers[7] used aluminum chloride both in the presence and in the absence of anisole to deprotect benzyl esters. In that work, they observed that cleavage of the Boc protective group also occurred, resulting in yields up to 88% of the doubly deprotected antibiotic. Building on that discovery, we have adapted this procedure for the facile, quantitative removal of the Boc group during solid-phase oligonucleotide synthesis. Although DNA is not stable to high concentrations of this reagent, it is stable to dilute solutions that are sufficient for Boc removal. Anisole is essential to the reaction because thymidine is unstable to $AlCl_3$ unless anisole is also present (see Table 1).

Table 1. Efficacy of Boc Removal and Stability of Thymidine to Different Concentrations of Aluminum Chloride and Anisole.

[$AlCl_3$] (mM)	[Anisole] (mM)	Time (min)	Boc Removal (%)[8]	Loss of Thymidine (%)[9]
20	370	1.0	20	-
50	370	1.0	80	-
50	90	1.0	80	-
50	370	2.0	>97	-
66	275	1.0	86	<3
100	370	1.0	NA	25
100	-	1.0	NA	68

In a sample deprotection, aluminum chloride (67 mg; 0.50 mmol) and anisole (0.40 mL; 3.7 mmol) were dissolved in 10 ml of CH_2Cl_2: CH_3NO_2 (2:1 v/v).[10] A portion of the light maroon solution was then manually forced at a rate of 1 mL per min through the column containing the resin-bound oligonucleotide. The resin was then washed with water (2.0 mL) and CH_3CN (1 mL). At this point the oligonucleotide was deprotected and cleaved from the support with ammonium hydroxide.

For internal incorporation of the aminonucleoside, automated synthesis can be resumed after the wash with acetonitrile, generating of a phosphoramidate linkage. Figure 1 illustrates the monomeric composition of a trimer containing an internal 5'-amino-5'-deoxythymidine after enzymatic digestion. In addition to their use as substrates for selection experiments,[11] amine-containing oligonucleotides also show promise as potential antisense therapeutics.[12] The method described here can both facilitate the synthesis and, because it is compatible with each of the bases, increase the versatility and variety of these oligonucleotides. Thus, the Boc group can now be used with oligonucleotides of mixed sequence and can be readily removed without loss of purine bases or modification of the pyrimidine bases. The combination of $AlCl_3$ and anisole is a good alternative to protic acids for removal of the Boc group during solid-phase synthesis.

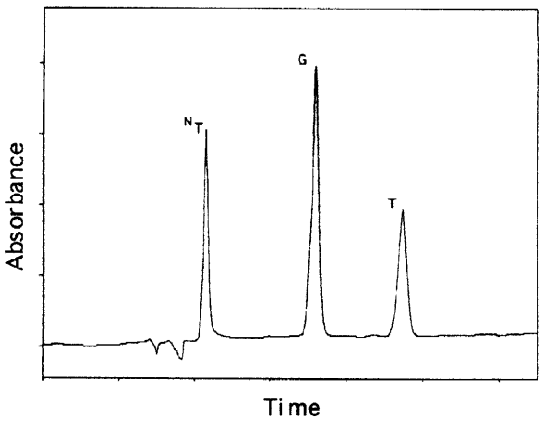

Fig. 1. HPLC trace of the digested trimer T^NTG.

ACKNOWLEDGMENTS

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6. To potentially minimize the reactivity of bromocatecholborane with adenosine while maintaining its ability to remove the Boc group, we attempted to poison the reagent both with pyridine and imidazole. Loss of adenosine was still evident.

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8. Boc removal was assayed by quantitative HPLC analysis. A resin-bound dimer composed of *N*-Boc-5'-amino-5'-deoxythymidine and thymidine (^{BocN}TT) was synthesized and exposed to $AlCl_3$/anisole while still attached to the resin. After NH_4OH deprotection and cleavage, the dimer was digested enzymatically. The percent deprotection was measured as the amount of 5'-amino-5'-deoxythymidine relative to thymidine. No percents are given for Boc removal from ^{BocN}TT with aluminum chloride at 100 mM due to the instability of the nucleoside at that concentration. It is clear that no Boc removal was due to incubation with NH_4OH. The Boc group was found to be stable to this reagent for one week at 55° C.

9. Loss of thymidine was assayed by quantitative HPLC analysis using the tetramer $^{5'}$TCAG$^{3'}$. Immediately following automated synthesis, a small fraction of the resin-bound tetramer was removed. The larger portion was exposed to $AlCl_3$/anisole. Both portions were then deprotected and cleaved with NH_4OH and then digested enzymatically. The percent loss of thymidine was determined by comparing the ratio of thymidine to the other nucleosides, both in the tetramer that had been exposed to $AlCl_3$ and the control tetramer.

10. Nitromethane (>99%) was used for the reactions described in Table 1. The use of lesser grade nitromethane (>95%) was found to result in lower yields during deprotection.

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