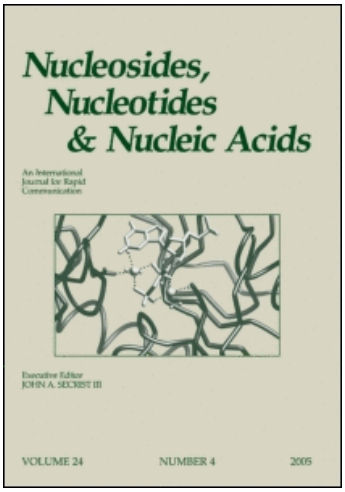

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AN IMPROVED DEPROTECTION PROCEDURE OF AMINE-CONTAINING OLIGONUCLEOTIDES FROM ACRYLONITRILE MODIFICATION

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ABSTRACT: Alkylation of alkylamine by acrylonitrile via Michael addition[1] occurred when standard ammonium hydroxide deprotection was used. The alkylation was greatly reduced using an improved two-step procedure when BCE phosphotriester protecting groups were selectively removed with t-butylamine prior to ammonium hydroxide deprotection.

For oligomers containing the N-trifluoroacetyl protected alkylamine [2] a two-step procedure was used: first, the support-bound oligomer was treated with t-butylamine in acetonitrile (1:4, v/v) for 1h at 20°C [3], followed by standard ammonium hydroxide deprotection.

The effectiveness of this procedure was investigated with two oligomers: Seq1: 5'-LCA CTT CAC TTT CTT TCC AAG AG-3'; and Seq2: 5'-AAG TAC GAC AAC CAC ATC TTT TTL TTT TTL TTT TTL TTT TTL T-3'; where L= N^4-(6-aminocaproyl-2-amino-ethyl)-5-methyl-2'-deoxycytidine [2]. Figure 1 shows the HPLC of enzymatic digestion [4] of Seq1 deprotected with either standard method (FIG.1A) or two-step procedure (FIG.1B).

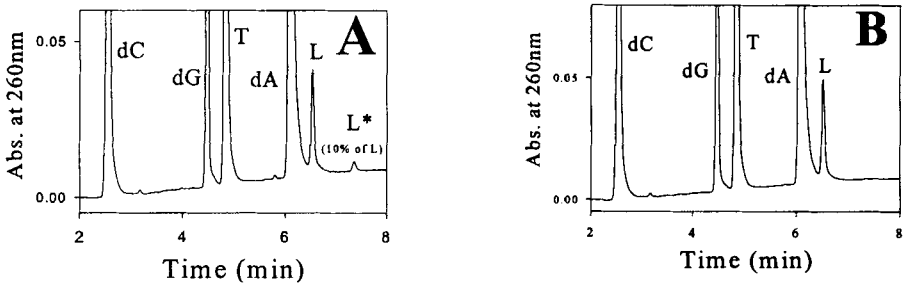

FIG.1. HPLC of enzymatic digestion of Seq1. Rainin Microsorb-MV C18 column, 4.6x100mm; Buffer: 30 mM ammonium acetate with 5% acetonitrile (v/v) at pH 4. Running method: isocratic at 0.8 ml/min.

A slower eluting peak (L*) was found in FIG.1A and was identified as acrylonitrile adduct of L [5]. An authentic sample was prepared and isolated by reacting L with acrylonitrile in ammonium hydroxide and it was found to co-elute with L* on HPLC.

This type of modification is especially problematic for oligomers with multiple alkylamines. A rather heterogeneous product mixture resulted from the derivatization of Seq2 with Bodipy FL NHS esters (Molecular Probes, OR) when the oligomer was deprotected under standard conditions (FIG. 2A), whereas FIG. 2B shows a much improved labeling of all four amines (4x peak) when Seq2 was deprotected with the new procedure.

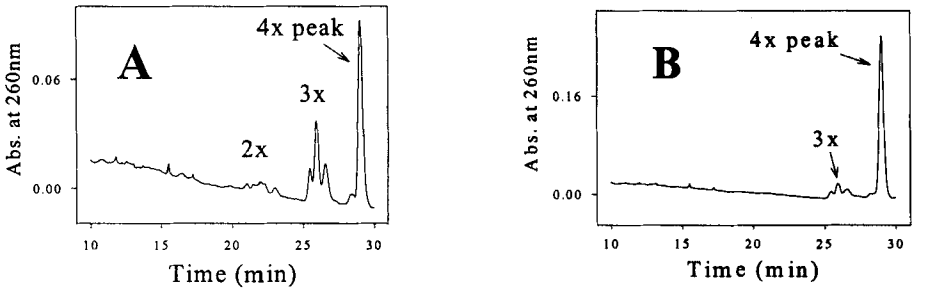

FIG.2. HPLC of Seq2 derivatized with BODIPY FL reactive dye. Same column as in FIG.1.; Buffer A: 0.1M triethylammonium acetate with 5% acetonitrile (v/v), pH 7.5; Buffer B: 100% acetonitrile. Running method: 0 to 30% B in 30 min at 1.2 ml/min. Peaks were assigned from a reaction of Seq2 with limiting amount of the same dye (data not shown).

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5. L*: ESI MS, MW calcd $C_{21}H_{34}N_6O_5$ 450.54, found 450.2; ^{1}H-NMR (CD_3COD) 1.3 (m, 2H), 1.4 (t, 2H), 1.5-1.6 (m, 4H), 1.95 (s, 3H), 2.1-2.3 (m, 4H), 2.55 (t, 2H), 2.85 (t, 2H).