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Anchor For One Step Release of 3′-Aminooligonucleotides from a Solid Support

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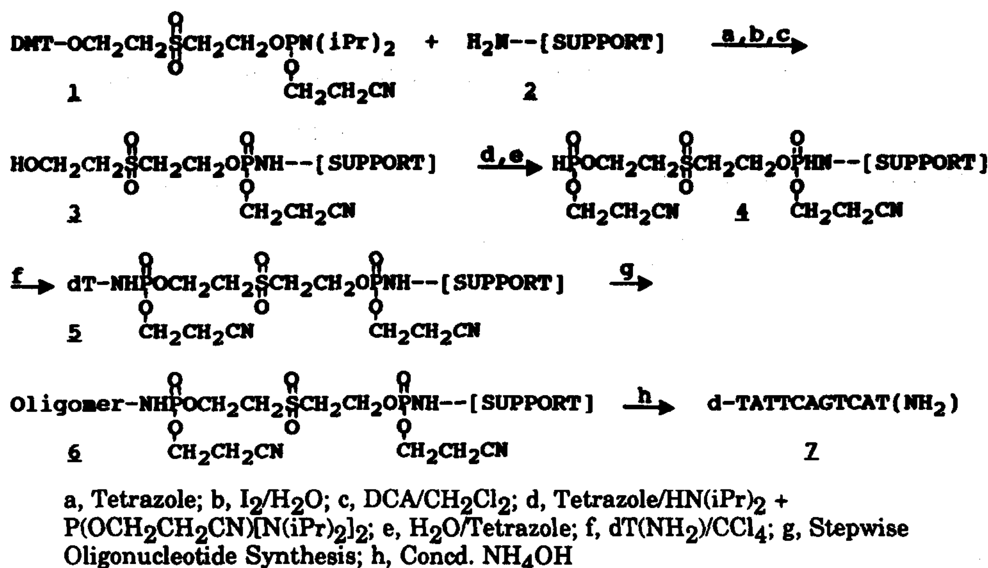
Abstract: A linker is described that permits covalently bound aminooligonucleotides to be liberated from a solid support by one step treatment with ammonium hydroxide.

Terminal amino groups in oligonucleotides serve as sites for attaching fluorescent labels, peptides, or other oligonucleotides, as groups for stabilizing hybridization in diagnostic and antisense applications, and as inhibitors of exonucleases. Recently we described a two step procedure for synthesis of 3′-aminooligonucleotides involving release of products from a solid support by successive treatment with ammonium hydroxide and aqueous acetic acid.[2] While convenient and effective for synthesis of many oligomers, the method requires that the polymeric products be stable both to concentrated ammonium hydroxide and to aqueous acetic acid (18-20 h). We describe here an anchor that permits a polymer linked through a terminal amino group to a solid support to be released in one step by treatment with ammonium hydroxide. The method should be particularly useful in the solid phase synthesis of amino terminal oligonucleotide analogues that contain functional groups sensitive to acetic acid.

The chemistry is outlined in chart 1. Condensation of a standard CPG solid support, 2, with 5′Phosphate-ON™ reagent (1, Cruachem, Inc.) followed by oxidation and detritylation affords support 3, which on phosphitilation and hydrolysis yields a H-phosphonate derivative, 4. A 3′-aminonucleoside is linked to the phosphorus by oxidative coupling using carbon tetrachloride, and the anchored nucleoside, 5, is extended by conventional phosphoramidite chemistry. Treatment with concentrated ammonium hydroxide then liberates the unprotected 3′-aminooligonucleotide. The cleavage presumably proceeds by ß-elimination initiated at α hydrogen atoms of the sulfone and cyanoethyl groups, followed by hydrolysis of an unstable phosphoramidate (compare the instability of 5′-phosphorylaminodeoxythymidine[3,4]).

Chart 1.



a, Tetrazole; b, I_2/H_2O; c, DCA/CH_2Cl_2; d, Tetrazole/$HN(iPr)_2$ + $P(OCH_2CH_2CN)[N(iPr)_2]_2$; e, H_2O/Tetrazole; f, $dT(NH_2)/CCl_4$; g, Stepwise Oligonucleotide Synthesis; h, Concd. NH_4OH

To demonstrate this approach, we showed that 3′-amino-3′-deoxythymidine could be efficiently loaded on and cleaved from an inert solid support (see Procedure section). A loaded support was then used for the synthesis of d-TATTCAGTCAT(NH_2) (7), an oligomer prepared previously _via_ the anchor requiring both ammonium hydroxide and acetic acid treatment for cleavage.[2] Reversed phase HPLC profiles for the newly prepared DMT derivative and oligomer 7 are shown in Figure 1A, 1B. The chromatographic properties of these materials and the Tm value (34°C, 0.1 M Na^+, 15 mM phosphate buffer pH 7.05) for dissociation of the complex formed from 7 and a complementary oligonucleotide were identical to those for this 3′-aminooligonucleotide prepared by the previously described method.[2] In further confirmation of the presence of the amino group, compound 7 was conjugated to fluorescein (~90% conversion) by treatment with excess FITC. This derivative (see Figure 1C for RP HPLC profile; λ_{max} 262, 462, 488 nm; λ_{min} 242, 320 nm) likewise proved to be identical to the fluorescein product reported previously[2].

It may be noted that attempts to use a succinyl or an oxalyl anchor in preparing 3′-amino-nucleoside derivatives were unsatisfactory. No aminodeoxythymidine was recovered when a sample of dT(NH)-succinyl-CPG was treated with concentrated ammonium hydroxide at 55° for 16 h. Under the same conditions a sample of dT(NH)-oxalyl-CPG[6] afforded some 3′-amino-3′-deoxythymidine but the major product was a nucleoside that coeluted with thymidine (see Figure 1D).[7]

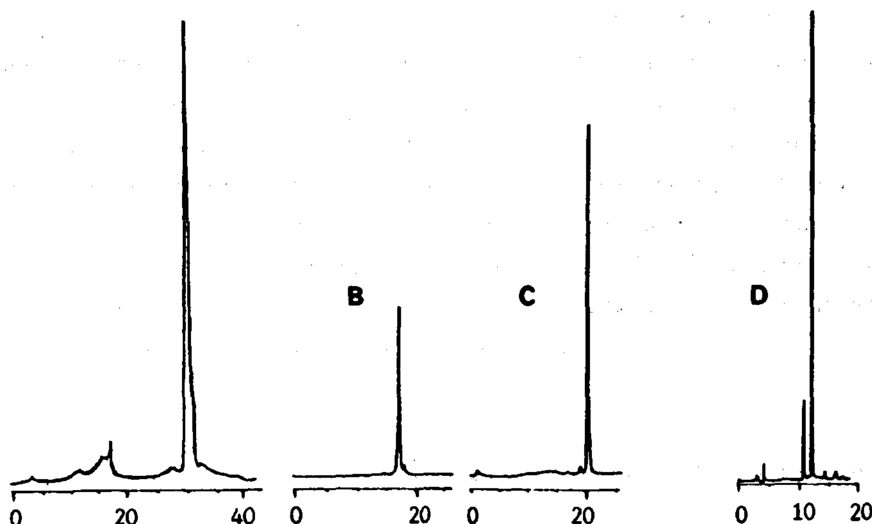


Figure 1. Reversed phase HPLC profiles (Dionex Hypersil ODS column, 5 μ, 4.6x200 mm, 1%/min. gradient of CH_3CN in 0.03 M Et_3NH^+ OAc^- buffer, pH 7.05). (A) crude d-DMT-TATTCAGTCAT(NH_2); (B) d-TATTCAGTCAT(NH_2) obtained by detritylation of main fraction from A; (C) fluorescein derivative of product from B; (D) products from cleavage of dT-oxalyl-CPG with NH_4OH. The minor product eluting at 10.6 min corresponds to dT(NH_2); the major product eluting at 12 min coelutes with dT.

This new anchor is readily constructed from commercially available materials. It should be applicable in solid supported synthesis of a wide variety of amine derivatives in addition to aminooligonucleotides. With respect to the release mechanism, the anchor has similarities to the Fmoc group. A novel feature is that the amine is ultimately released by dephosphorylation rather than the decarboxylation characteristic of cleavage of Fmoc, carbobenzoxy, and related protecting groups.

Procedures. LCAA controlled pore glass (500 mg, 500 Å, 80-120 mesh, Sigma) was placed in a syringe and treated (3 min) with Phosphate-ON™ reagent (Cruachem, 0.1 M in CH_3CN, 1.0 ml) and tetrazole (0.4 M in CH_3CN, 1 ml); then the solid was washed (CH_3CN), oxidized with iodine (0.15 M in pyridine/THF/H_2O, 1 min), deprotected (2.5% DCA in CH_2Cl_2), and dried in vacuo. The loading amounted to ~38 μmole/g of support as judged by the DMT test. Phosphitilation was effected with a solution of ß-cyanoethyl-bis-diisopropylaminophosphine (320 μl, Aldrich) and diisopropylammonium tetrazolide (170 mg) in CH_2Cl_2 (10 ml), 1.5 h at room temperature, and the product was converted to the H-phosphonate by hydrolysis (5 min) with 3 ml of 0.4 M

tetrazole in CH_3CN/H_2O (5/1, v/v). After washing (CH_3CN, CH_2Cl_2) and drying (vacuum desiccator), the solid was treated with 2 ml of 0.2 M 3'-amino-3'-deoxythymidine in C_5H_5N/CCl_4, 1/1, v/v, 45 min., washed well, dried, and stored in a vacuum desiccator at +4°C. To determine the loading and as a test of cleavage conditions, a sample (10 mg) was treated with concentrated ammonium hydroxide (2.5 h, 55°C). 3'-Amino-3'-deoxythymidine (0.31 A_{260} units, 0.35 µmole, corresponding to ~35 µmole/g support) was recovered from the ammoniacal solution as the sole product: RP HPLC retention time (conditions as in Figure 1), 10.6 min; Rf for TLC on silica gel plates in i-PrOH/NH_3/H_2O 7/1/2 v/v/v, 0.65; positive ninhydrin test.

The oligonucleotide synthesis was carried out on a 1 µmole scale in a conventional manner using ß-cyanoethyl phosphoramidite reagents and a Cyclone MilliGen/Biosearch synthesizer.

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References and Footnotes.

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6. 5'-O-Trityl-3'-amino-3'-deoxythymidine was attached to a CPG support analogously to 5'-O-dimethoxytritylthymidine (Alul, R.H.; Singman, N.; Zhang, G.; Letsinger, R.L. *Nucleic Acids Research*, **1991**, *19*, 1527-1532) and the trityl group was removed with dichloroacetic acid (3% in CH_2Cl_2, 30 min, RT).
7. Aminodeoxythymidine is stable under these conditions. Although the cleavage product which coelutes with thymidine was not further characterized, it may be noted that thymidine could form from the oxamide derivative by a double inversion at the 3' carbon atom involving, first, displacement of an amide fragment by the 2-oxygen atom of an ionized thymine residue, then, backside attack on the resulting anhydro nucleoside by hydroxide ion.

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