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Elimination of Transamination Side Product by the Use of dC^{Ac} Methylphosphonamidite in the Synthesis of Oligonucleoside Methylphosphonates

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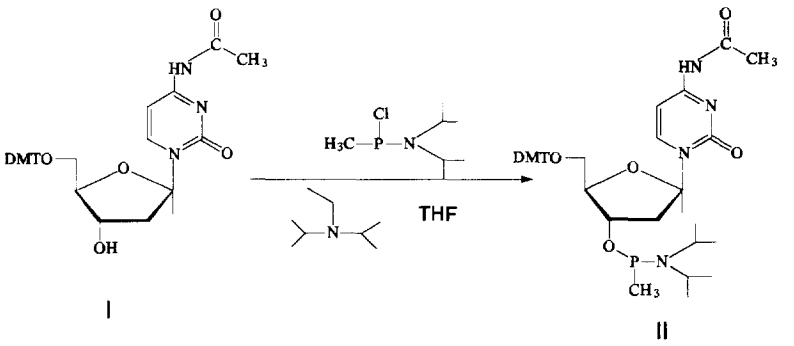
Abstract: The transamination side product formed by the use of dC^{bz} or dC^{ibu} methylphosphonamidite upon treatment with ethylenediamine has been eliminated by the use of dC^{Ac} methylphosphonamidite. The synthesis and characterization of DMT dC^{Ac} methylphosphonamidite is described.

Oligonucleoside methylphosphonates, which contain a methylphosphonate backbone in the place of a phosphodiester backbone present in the normal oligonucleotides, are among the widely studied modified oligonucleotides as potential therapeutic agents.[1-3] They satisfy the criteria of nuclease resistance, cell membrane permeability and affinity to complementary nucleic acid strands. They are usually synthesized by a solid phase methodology by using chemistries similar to those employed in the synthesis of normal oligonucleotides. The major difference, however, is in the post synthesis processing. Where as normal oligonucleotides are usually cleaved and deprotected with ammonium hydroxide[4] or methylamine,[5,6] their use for the cleavage and deprotection of oligonucleoside methylphosphonates results in significant degradation of the methylphosphonate backbone.[7,8]

In order to avoid the undesirable degradation, Miller et al.[9] have developed ethylenediamine/ethanol as an efficient reagent which substantially reduces the chain cleavage. Under these conditions, however, ethylenediamine produced a significant amount of transamination product upon reaction with the commonly used N^4-benzoyl deoxycytidine(dC^{bz}).[10] In order to reduce this undesirable transamination product, Hogrefe et al[11-13] have employed N^4-isobutyryl deoxycytidine methylphosphonamidite (dC^{ibu}). Although use of dC^{ibu} derivative has substantially reduced the transamination product formation, a small amount is still formed (discussed later).

We have developed before a fast oligonucleotide cleavage and deprotection chemistry[5,6] employing methylamine or methylamine/ammonium hydroxide. In order to avoid the transamination product formed with dC^{bz} and dC^{ibu} upon reaction with methylamine, we have successfully developed N^4-acetyldeoxycytidine (dC^{Ac}) phosphoramidite. This dC^{Ac} derivative was synthesized in good yields with high purity and it was found to be a very stable molecule both during shelf storage and during use on automated DNA synthesizers.[14] Also, use of dC^{Ac} phosphoramidite did not require any program or procedure changes during DNA synthesis. We now believe that the use of

temperature and the products were analyzed by reverse phase HPLC.[15] As figure 1 shows dC^{Ac} nucleoside did not show detectable transamination side product where as dC^{ibu} and dC^{bz} showed N^4-(2-aminoethyl) deoxycytidine side product. Encouraged by this finding, we have synthesized 5'-DMT dC^{Ac} 3'-methylphosphonamidite(II) as shown below.



5'-DMT-N^4-acetyldeoxycytidine[5](I, 11.42 gm, 20 mmoles) was dried by successive coevaporations with pyridine, toluene and THF. The dried residue was dissolved in dry THF (100 ml) and redistilled N,N,N-diisopropylethylamine (17 ml, 80 mmoles) was added, followed by the dropwise addition of methylmonochloro-N,N-diisopropylphosphonamidite (10 ml) under argon over a period of 5 minutes. After 5 hours of stirring, the reaction mixture was diluted with ethylacetate (250 ml), washed with 10% $NaHCO_3$ solution (2 x 200 ml) and dried over Na_2SO_4. The crude material was dissolved in ethylacetate and chromatographed on a silica gel column. It was evaporated to dryness and was further dried under high vacuum for 5 hours to yield methylphosphonamidite, II (8.6 gm, 60% yield). M.p. 100-110°C. ^{1}H-NMR ($CDCl_3$): δ 0.86-1.27 (m, 15 H, P-C$\underline{H}_3$ and C$\underline{H}_3$ iPr), 2.25 (s, 3H, COC$\underline{H}_3$), 2.28 and 2.70 (2m, 2H, $C_{2'}$ C$\underline{H}_2$), 3.45 (m, 4H, $C_{5'}$ C$\underline{H}_2$, 2 x C$\underline{H}$ iPr), 3.80 (s, 6H, 2 x OC$\underline{H}_3$ of DMTr), 4.15 (m, 1H, $C_{4'}$ $\underline{H}$), 4.50 (m, 1H, $C_{3'}$ $\underline{H}$), 6.22 (2t, 1H, $C_{1'}$ $\underline{H}$), 6.81-7.43 (m, 14 H, $C_5\underline{H}$ and aromatic protons of DMTr), 8.25 (2d, 1H, $C_6\underline{H}$), and 9.70 (br, s, 1H, N$\underline{H}$CO). ^{31}P-NMR ($CDCl_3$): δ 121.15 ppm and 119.65 ppm. Elemental analysis: Calcd. for $C_{39}H_{49}N_4O_7P.2H_2O$ (752.78): C, 62.22; H, 7.10; N, 7.44; P, 4.11 Found: C, 62.40; H, 7.57; N, 7.02; P, 4.30. HPLC: retention time of 15.34 corresponding to two diastereoisomers (99.35% purity); Conditions: C_{18} Microsorb column (Rainin) 5 μ particles, 4.6 mm x 25 cm. Bottle A: 0.1 M Ammonium acetate (pH 6.9); Bottle B: Acetonitrile. Program: Flow rate 1 ml/min. 0-20 min at 80%B.

In order to perform comparative evaluation of transamination side product formation, d(CT) methylphosphonate dimer was synthesized by coupling dC^{Ac} or dC^{ibu} or dC^{bz} methylphosphonamidite with T---CPG using standard coupling conditions. After the synthesis, the dimers were cleaved and deprotected with ethylenediamine/ethanol (1:1) for 7h at room temperature. After evaporation, the products were analyzed by reverse phase HPLC.[15] As the data in figure 2 show, use of dC^{Ac} methylphosphonamidite appears to have eliminated the formation of transamination side product. We observed similar result with the d(TC) dimer synthesized by coupling T methylphosphonamidite to dC^{bz}, dC^{ibu} or dC^{Ac}--CPG solid supports.

Figure 1. Transamination product dC* formed by ethylenediamine (EDA).
dC*: N^4-(2-aminoethyl) deoxycytidine.

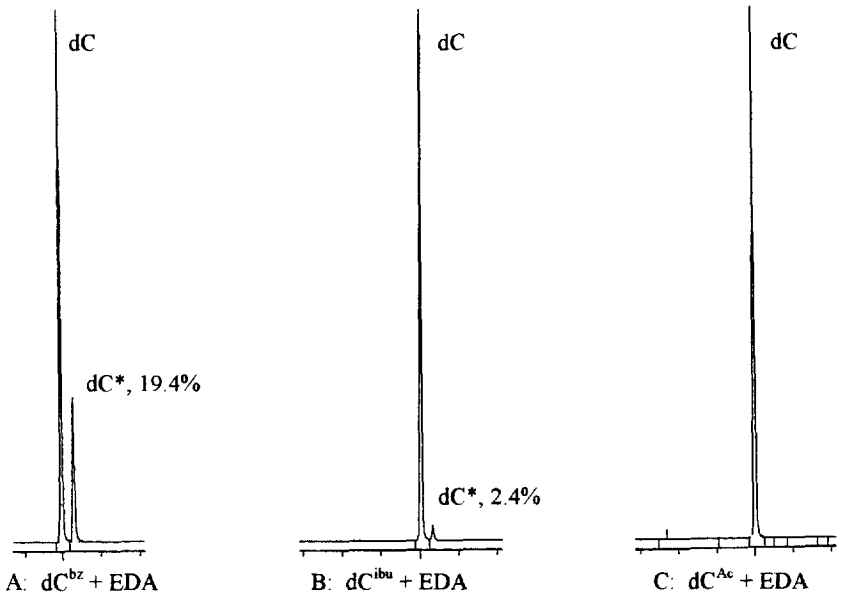
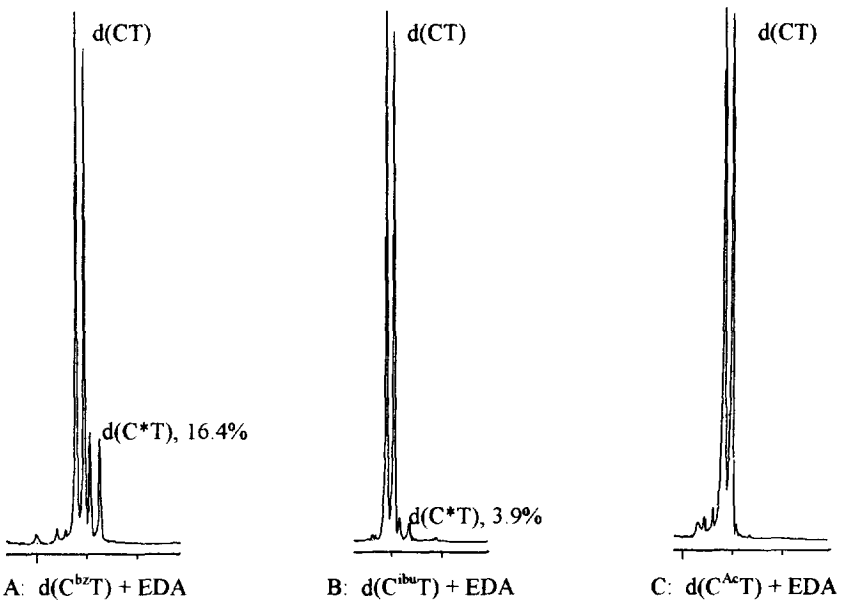


Figure 2. Transamination product formed by ethylenediamine with methylphosphonate dimers. Two peaks are due to the diastereomers resulting from the chiral phosphorus center.



In order to further confirm our findings a dC_{10} oligonucleoside methylphosphonate was synthesized by using dC^{Ac} or dC^{ibu} or dC^{bz} methylphosphonamidite and the corresponding dC solid supports. Since Reverse phase HPLC of dC_{10} did not provide the necessary resolution to detect the transaminated species, the oligomer was digested with aqueous piperidine[10] and analyzed by reverse phase HPLC.[15] In the case of dC^{bz} containing oligomer, we observed a total of four peaks, the first two corresponding to dC and dC methylphosphonate and the next two peaks accounting for 26.2% corresponding to the transaminated species. dC^{ibu} containing oligomer showed the same four peaks, but the transaminated species were reduced to 5.0%. As expected dC^{Ac} containing oligomer showed only two peaks corresponding to dC and dC methylphosphonate and did not show the transaminated species. Considering the therapeutic potential of oligonucleoside methylphosphonates we believe that it is important to avoid the side product formation as much as possible.

In conclusion, we have developed dC^{Ac} methylphosphonamidite[16] to eliminate the transamination side product observed with the use of dC^{bz} and dC^{ibu} methylphosphonamidites.

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14. C^{Ac} phosphoramidite is commercially available from Beckman Instruments and Glen Research under the name 'Ultra*FAST* cleavage and deprotection system'.
15. HPLC conditions:C_{18} Microsorb column (Rainin) 5 µ particles, 4.6 mm x 25 cm was used. Bottle A: contained 0.1 M ammonium acetate, pH 6.9; Bottle B: contained HPLC grade acetonitrile. Flow rate was 1ml/min. 0-20 mins gradient to 15% B, 20-25 mins gradient to 25% B, 25-27 mins gradient to 50% B, 27-30 mins to 50% B, 30-35 mins to 0% B.
16. Reddy, M. P., Farooqui, F.; Hanna, N. B. Patents pending.

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