Synthesis of oligonucleotides using the 2-(levulinyloxymethyl)-5-nitrobenzoyl group for the 5'-position of nucleoside 3'-H-phosphonate and -H-phosphonothioate derivatives

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ABSTRACT
Synthetic studies on phosphodiester, phosphorothioate, and phosphorodithioate-linked oligonucleotides in terms of 2-(levulinyloxymethyl)-5-nitrobenzoyl (LMNBz) group as the base-labile protecting group for the 5'-hydroxyl groups of nucleoside 3'-H-phosphonate and -H-phosphonothioate derivatives, are described.

INTRODUCTION
The current methodology of the automated solid-phase approach to oligonucleotides on controlled pore glass (CPG) support apparently seems to be used successfully, but has been hampered by a series of problems. In the case of oligodeoxyribonucleotide synthesis, the depurination reaction occurs inevitably under the acidic conditions used for the removal of the 5'-O-(4,4'-dimethoxytrityl) (DMTr) protecting group. In the case of oligoribonucleotide synthesis, on the other hand, the bulky tert-butyldimethylsilyl (TBDMS) protecting group is widely used for the 2'-hydroxyl group of ribonucleosides in place of the tetrahydropyran-2-yl (Thp) group, which is somewhat affected by the removal of the 5'-O-DMTr group under acidic conditions. The 2'-O-TBDMS group, however, brings about an unfavorable steric effect on coupling reactions of the ribonucleoside phosphoramidite units. Consequently, various protecting groups for the 5'-hydroxyl groups of ribonucleoside and 2'-deoxyribonucleoside 3'-phosphoramidites have been reported, as exemplified by the levulinyl group and the modified 2-hydroxymethyl-benzoyl groups, which are removable under basic conditions. The former was characterized by facile unmasking by 0.5 M hydrazine hydrate in 1:4 acetic acid - pyridine at room temperature for 2 min, although the yield for its introduction was unsatisfactory (30-6% for 2'-O-Thf-U, -C*, A*Bz, G). Based on the chemistry of these protecting groups, we have also reported the utility of the 2-(levulinyloxymethyl)-5-nitrobenzoyl (LMNBz) group, which can be introduced with a higher regioselectivity and is still removable under similar basic conditions. The protecting reagent, 2-(levulinyloxymethyl)-5-nitrobenzoic acid (1), was easily prepared from phthalide. The LMNBz group has been introduced at the 5' position of 2'-O-Thf-ribonucleosides (2) and 2'-deoxyribonucleosides (3) by treatment with 1 (1.1 equiv) in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCI) (2.2 equiv) in pyridine at room temperature with yields over 60% regardless of the structure of the nucleosides (Scheme 1). Moreover, the LMNBz group has been removed by a consecutive treatment with 0.5 M hydrazine hydrate in 1:4 acetic acid - pyridine, and with 0.5 M imidazole in acetonitrile. Using the LMNBz protecting group, syntheses of both DNA- and RNA-type oligomers have been performed efficiently on CPG support by the phosphoramidite approach.

RESULTS AND DISCUSSION
5'-O-LMNBz-nucleoside 3'-H-phosphonates (5, X=O) were prepared by treating 5'-O-LMNBz-nucleosides (4) with tris(l,2,4-triazol-1-yl)phosphite (4-5 equiv) and subsequent hydrolysis with aqueous triethylammonium bicarbonate by the method of Froehler. The H-phosphonate and H-phosphonothioate approaches were conducted, and the results are described herein.
the LMNBz group of the nucleoside (6) linked to CPG support, the \(H\)-phosphonate monomers (5, \(X=O\)) were coupled to the growing oligomer (7) supported in the presence of pivaloyl chloride as a condensing agent. After completion of the \(H\)-phosphonate oligonucleotide assembly, the LMNBz group was removed, followed by oxidation with \(I_2/H_2O\) or elemental sulfur to yield the desired phosphodiester or phosphorothioate-linked oligonucleotides, cleavage of oligomers from the support, and deprotection. Phosphodiester (TpT, TpTpT, and TpTpTpT) and phosphorothioate-linked (TpsT and TpsTpsT) oligonucleotides were synthesized, and HPLC tracings of the each of the resulting mixtures are shown in Figs. 1a-e, respectively.

The successful synthesis of phosphodiester and phosphorothioate-linked oligonucleotides by the above described approach motivated us to extend our studies to the synthesis of phosphorodithioate-linked oligonucleotides, which is expected to be one of the antisense probes. \(^{10}\)

\(5'-O\-LMNBz\)-nucleoside 3'-\(H\)-phosphonothioates (5, \(X=S\)) were prepared by treating \(S'-O\)-LMNBz-nucleosides (4) with triethylammonium phosphinate in the presence of pivaloyl chloride along with subsequent treatment with elemental sulfur by the method of Stawinski. \(^{11}\) The \(H\)-phosphonothioate monomers (5, \(X=S\)) were used for the assembly of phosphorodithioate-linked oligonucleotides through the reaction cycle, as described above for the synthesis of phosphorothioate-linked oligonucleotides, using diethyl phosphorochloridate as a condensing agent. \(^{12}\)

Starting with 6 (B=T), the synthesis of Tps2Tps2T was efficiently performed (Fig. 1f).

REFERENCES


