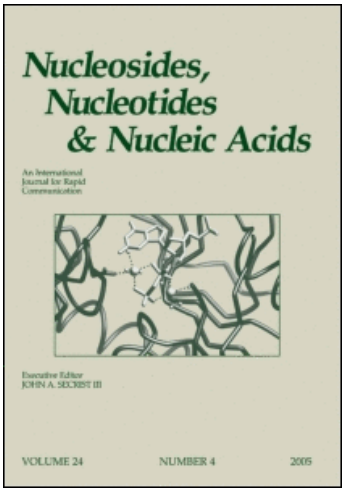

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Solid Phase -Nucleotide Links Cleavable by Mild Acid Treatments: Phosphoramidates, Phosphoesters and Ethers

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SOLID PHASE -NUCLEOTIDE LINKS CLEAVABLE BY MILD ACID TREATMENTS: PHOSPHORAMIDATES, PHOSPHOESTERS AND ETHERS

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Abstract: Acid sensitive links between nucleotides and a polystyrene-trialkoxybenzhydryl resin allow for the release of protected oligonucleotide fragments into solution

Solid phase - nucleotide links cleavable by mild acid in combination with alternative base and sugar protections (like e.g. the nitrophenylsulfenyl and the fluorenylmethyloxycarbonyl group respectively) might contribute to a gain in flexibility of synthesis designs in the field of chemically modified nucleic acids. We evaluated the properties of a limited number of ether, phosphoramidate and phosphoester links with respect to their lability towards mild acid treatments. Our report focuses on the use of a phosphoester link with a trialkoxybenzhydryl-polystyrene resin (1) originally designed for peptide synthesis (Rink-Resin). The three types of links mentioned above are each suited for conceptually different tasks. Ether linkages bind the sugar moiety of the nucleoside. Deprotection leads to oligomers lacking terminal phosphate groups. Preliminary tests allowed us to directly functionalize an o-chlorotrityl resin normally used in peptide synthesis (2) with a 5'-Fmoc protected nucleoside (16 µMol/g). 10% acetic acid in dichloromethane quantitatively cleaves the ether linkage. Phosphoramidate linkages appealed to us as a potential means of synthesizing nucleic acids bearing a free terminal phosphate group. Phosphotriesters of the Rink-Resin are markedly acid labile and allow for the release of protected oligonucleotide fragments into solution, ready for further derivatization in the liquid phase (Fig. 1).

The Rink-Resin was functionalized using 5'-Fmoc-dT as starting material and applying conditions described in van Boom's triester approach (3). Fmoc could be cleaved almost immediately with 0.1 N DBU in aceto-

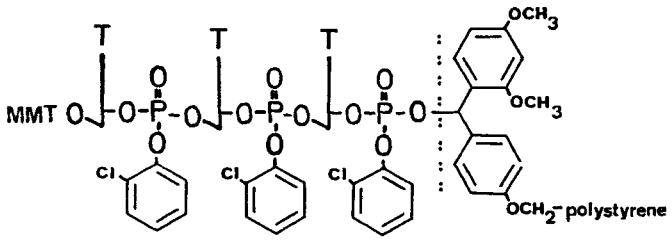

FIG. 1

nitrile+dichloromethane (DCM) (80+20). Loadings of 40 μMol/g were measured spectrophotometrically. Coupling procedures for chain elongation were carried out by the hydroxybenzotriazole phosphotriester approach (3). For terminal 5'-OH protection MMT was given the preference over Fmoc because it substantially increased the solubility of the protected fragment upon cleavage from the resin. MMT protection remained unaffected during the 5 min dilute acid treatment (10% acetic acid in DCM) Acidic washings were neutralized with triethylamine; The resin was rinsed with DCM+CH_3OH+triethylamine (90+5+5). The solubilized fragment was purified through a Sephadex® LH-20 column in methanol, yielding 64% of theory (negative ion FAB: m/z = 1532). A 3'-capped tetramer was obtained by reacting with 3'-tert.butyldimethylsilyl-dT in solution using standard coupling and deprotection procedures (sodium salt: negative ion FAB: M-Na = 1311.5).

We are also investigating the properties of a resin similar to 1 carrying a phosphoramidate functionality, i.e. a morpholine residue instead of the o-chlorophenyl residue. About a 35% release of the morpholino-protected nucleotide moiety may be obtained with 3% dichloroacetic acid in DCM. Such a derivative allows for complete deprotection of the terminal phosphate group. Cleavage rates are limited because of the concurrent elimination of the morpholine residue causing the resulting phosphodiester link to become completely stable against mild acid treatments.

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