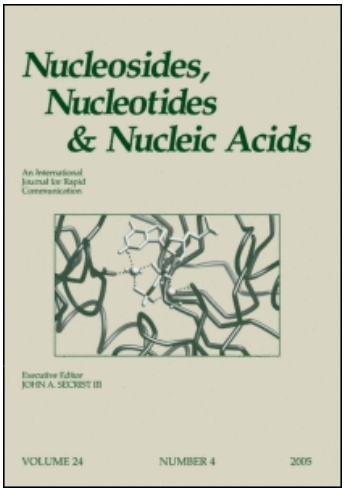

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o-CHLOROBENZOYL PROTECTED NUCLEOSIDE SUCCINATES FOR FUNCTIONALISATION OF THE SOLID SUPPORT USED IN OLIGORIBONUCLEOTIDE SYNTHESIS

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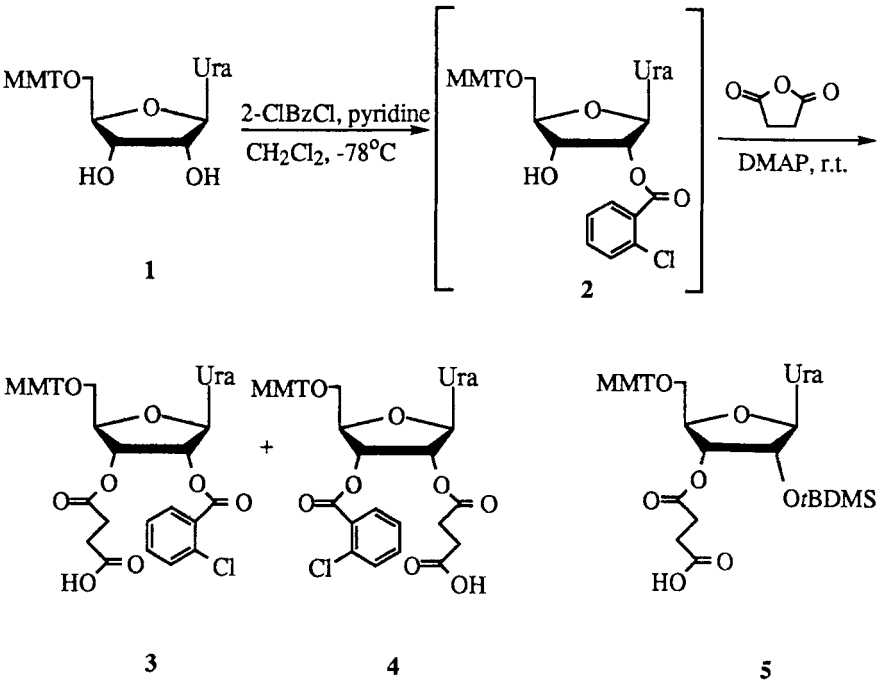
In the final stages of automated oligonucleotide synthesis the oligomer has to be cleaved from the solid support. This is usually carried out using ammonolysis since the 3'-end of the oligomer is most commonly attached to the support via a succinate ester linkage. The *t*-butyldimethylsilyl (TBDMS) group is currently the most widely used 2'-hydroxyl in RNA-synthesis and is used together with phosphoroamidites[1] as well as with H-phosphonates[2]. The nucleoside directly attached to the support, often carries the same TBDMS-protection on the secondary hydroxyl next to the succinate linker. The use of more labile acyl groups for N-protection in RNA-synthesis was suggested in reports where partial loss of the TBDMS groups during ammonolysis was detected[3,4]. This has since been introduced[5,6] and is now general practice. However, one can question if all oligomer will be released from the support under the milder ammonolytic conditions used to remove these more labile N-protecting groups.

We decided to compare release of oligomers having a TBDMS or *o*-chlorobenzoyl protected nucleoside succinate terminal linked to the solid support. This *o*-chlorobenzoyl (ClBz) group has recently been introduced as an alternative 2'-OH protection in H-phosphonate based synthesis of oligoribonucleotides[7] and the succinates **3** and **4** are conveniently made from **1** in a one pot procedure according to the Scheme.



Scheme

The supports used in the ammonolysis studies were made by synthesising oligouridylic acids (2'-O-TBDMS-Up)$_{20}$U on long chain alkylamine controlled pore glass beads (Pierce LCAA-CPG, 500 Å) functionalised with either a mixture of **3** and **4** or with **5** . A standard protocol for oligoribonucleotide synthesis with the H-phosphonate approach[8] was used. The rate of release of (U(Si)p)$_{20}$U from the two differently functionalised supports during ammonolysis in 32 % conc. NH_3 (aq)-ethanol (3:1) at room temperature (around 20 °C) was determined. Ammonia solution (1 ml) was added to the (U(Si)p)$_{20}$U-support and aliquots of 5 µl were withdrawn from the supernatant at different times. These samples were diluted to 1 ml in phosphate buffer (0.5M) and the UV-absorbance was measured at 257 nm.

The release of (2'-O-TBDMS-Up)$_{20}$U upon ammonolysis (Fig) is considerably faster with the support functionalised with **3** and **4** than with **5**, the rate difference being of more than an order of magnitude. With the support functionalised with the TBDMS-succinate **5** the oligomer is not completely

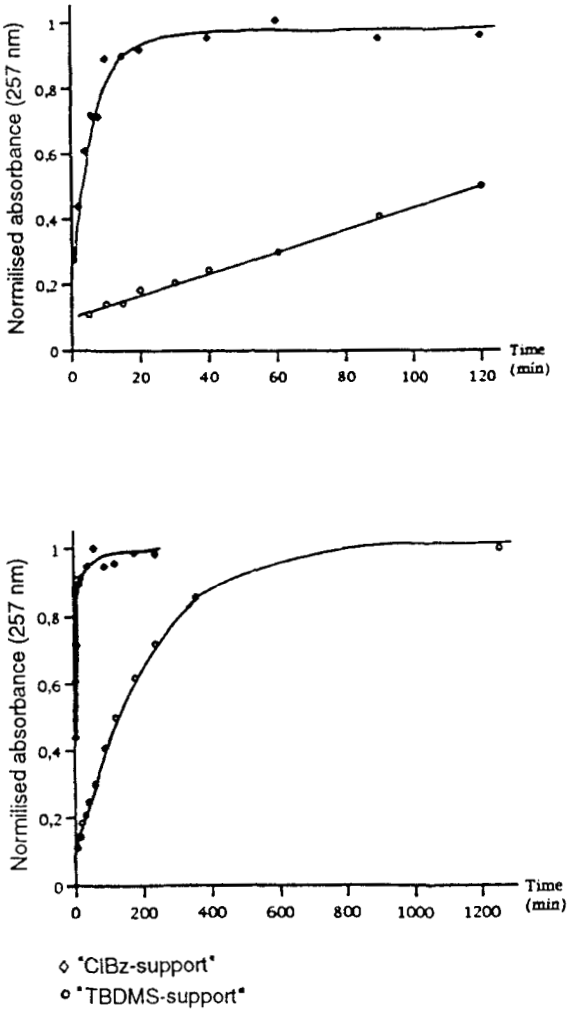


Figure Release of (2'-O-TBDMS-Up)$_{20}$U from LCAA-CPG functionalised with **3** and **4** ("ClBz-support") or with **5** ("TBDMS-support").

released within the time necessary for removal of the more labile N-protection commonly used today. This is not satisfactory since having made the precious RNA-fragment it is of course desirable to recover as much as possible without having to use an order of magnitude longer time than necessary for removal of other groups also with the risk of losing some of the TBDMS-groups and then possibly getting some cleavage of phosphodisters. When support functionalised with **3** and **4** is used the oligomer is completely released within

the time necessary for removal of the N-acyl groups. These succinates are better matched to the N-protection and thus clearly preferrable to TBDMS-protected succinate. A substantial further advantage of using the o-chlorobenzoyl protected succinates **3** and **4** is that the synthesis of them is a more convenient one pot procedure starting from nucleoside derivatives with both secondary hydroxyls unprotected, e. g., MMT-U (**1**).

The general procedure for synthesis of chlorobenzoyl protected succinates is as follows: The 5'-O- and N-protected nucleoside is treated with 2-chlorobenzoyl chloride (1.1 eq.) in CH_2Cl_2-pyridine, 19:1 at -78°C for 30 min to give the selectively mono 2'-chlorobenzoylated nucleoside. Succinic anhydride (1.1 eq.) and DMAP (2.2 eq.) is then added directly in the same reaction media and the reaction mixture is further stirred at room temperature for 24 h. An isomeric mixture of 2' or 3'-ClBz 2' or 3'-succinate derivatives is obtained due to the rapid isomerization of 2'-chlorobenzoylated nucleoside catalysed by DMAP. The product is purified using silica gel chromatography but the isomers are not separated since both may be equally well used for functionalisation of the solid support.

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