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An Orthogonal Solid Phase Support for the Synthesis of Oligonucleotides Containing 3'-Phosphates and Its Application in the Preparation of Photolabile Hybridization Probes

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Abstract: Photolabile oligonucleotide synthesis supports (**3**) can be used to prepare biopolymers containing 3'-phosphates in good yield. When **3** is prepared using Tentagel®, oligonucleotides can be deprotected using standard concentrated aqueous ammonia conditions without cleaving the biopolymer from the support. The support bound oligonucleotide can capture its complement, and discriminate between a one base pair mismatch. Following photocleavage, the respective duplex is isolable by gel electrophoresis. © 1998 Elsevier Science Ltd. All rights reserved.

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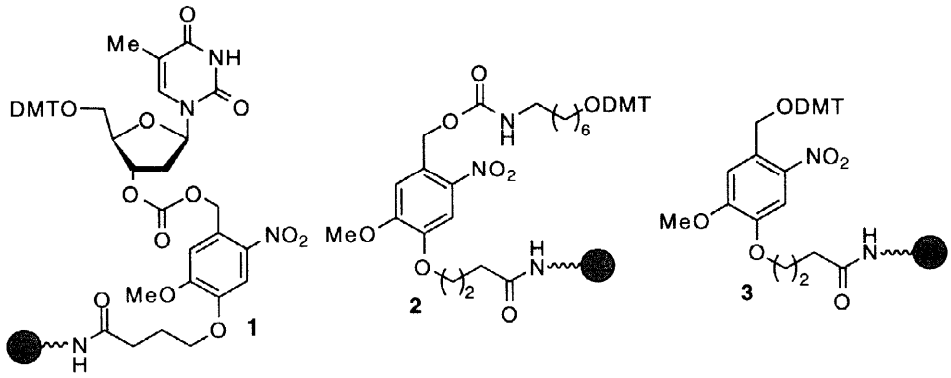
The *o*-nitrobenzyl photo-redox reaction was originally employed in solid phase synthesis more than 20 years ago.[1] There has been a surge of interest in using this venerable photochemical reaction in a range of solid phase synthesis applications.[2-4] The *o*-nitrobenzyl photo-redox process has also been useful in the development of a number of useful solid phase oligonucleotide synthesis supports that facilitate the synthesis of oligonucleotides containing alkaline labile lesions (e.g. **1**).[5,6] More recently, **2** has enabled the development of new, high yielding, convergent methods for the synthesis of oligonucleotide conjugates.[7]



This Letter is prompted by recent reports concerning the release of oligonucleotides containing 3'-phosphate termini.[8] While oligonucleotide synthesis reagents are available that enable one to introduce 3'-phosphate termini into fully deprotected oligonucleotides, solid phase supports that are stable to concentrated aqueous ammonia deprotection and/or produce protected oligonucleotides containing this functional group are

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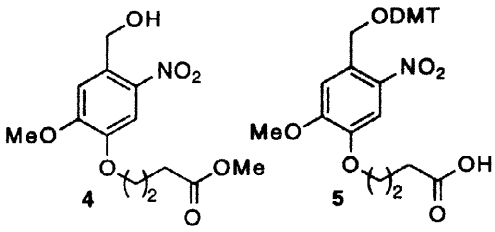
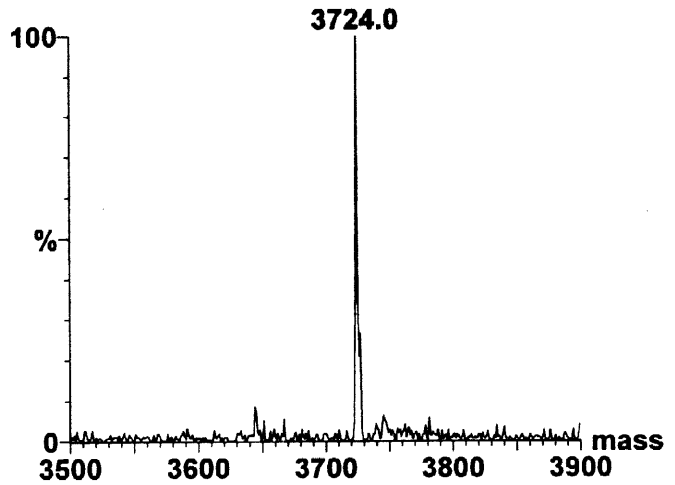
potentially useful for carrying out bioconjugation studies on protected oligonucleotides, and as solid phase bound hybridization probes.[7,9,10]



The requisite solid phase support (**3**) was readily prepared from previously reported **4**.[5] The free acid was activated *in situ*, and loaded on to long chain alkyl amine controlled pore glass support (LCAA-CPG) using only two equivalents of **5** per alkyl amine.[11] The absence of a nucleoside on the support enables one to utilize **3** to synthesize oligonucleotides of any sequence. Couplings on **3** were as efficient as on commercial resins (≥98%, as determined by dimethoxytrityl cation responses) using standard automated oligonucleotide synthesis cycles and commercially available phosphoramidites.[5] Oligonucleotides were cleaved from the support using a transilluminator (4 × 8 W lamps) at 365 nm for 2 h, conditions which have been shown to not damage the biopolymers.[5] The yields of photocleaved oligonucleotides from **3** could not be determined via the standard procedure developed in our group, because the *o*-nitrobenzyl-phosphate triester linkage is stable to aqueous ammonium hydroxide. Consequently, yields were determined relative to an identical oligonucleotide sequence prepared on commercially available succinato resin. The oligonucleotides were purified and isolated alongside one another, and absolute yields (shown below) were normalized based upon the respective dimethoxytrityl cation responses measured during the course of the respective automated syntheses. The integrity of the oligonucleotide was verified by electrospray mass spectrometry (Figure 1).



5'-d(ACG ATG AGT CCT)–O–P(O⁻)–O⁻
6 66 %

5'-d(T_{20})–O–P(O⁻)–O⁻
7 44 %

5'-d(TGA GGT TAC CCA CTC ACA GT)–O–P(O⁻)–O⁻
8 45 %

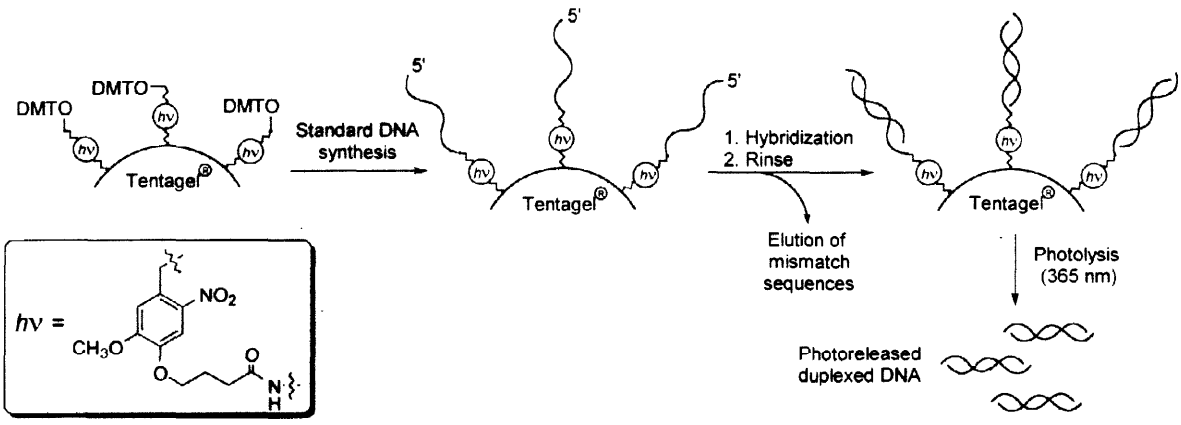
5'-d(AGG ACT CAT CGT)
9

5'-d(AGG ACT AAT CGT)
10

Figure 1. Electrospray mass spectrum of **6** (calcd. mass: 3724.4).

The stability of the linkage between the chromophore and oligonucleotide suggested that **3** would be useful for preparing hybridization probes. A significant advantage of using supports based upon **3** is that the duplexed material can be isolated following photolysis (Scheme 1).[10] In order to demonstrate this concept, an oligonucleotide with the sequence of **6** was prepared on Tentagel® using standard automated synthesis cycles and deprotection conditions.[12] Irradiation of the support bound, deprotected oligonucleotide as described above,

Scheme 1



yielded **6** in comparable yield to that obtained for photolysis of protected oligonucleotides. The use of support bound **6** as a hybridization probe was then demonstrated using radiolabeled oligonucleotides, complement **9** and single base mismatch **10**. Hybridization was carried out at 45 °C, in order to take advantage of the differential stability of the respective duplexes formed by support bound **6** with **9** and **10**.[13] Resin that was hybridized with **10** retained low levels of radiation (< 0.9%), that were not released upon heating to 85 °C, or upon photolysis (Figure 2). These observations led us to conclude that the small amounts of **10** retained by the resin bearing **6** are bound nonspecifically. In contrast, significantly larger amounts of **9** were retained by the resin. However, >60% of this radiolabeled material was released into the water washings upon photolysis, suggesting that **9** was bound specifically to support bound **6**. Further evidence for duplex formation between **6** and **9** was obtained following analysis of the cleaved material by nondenaturing polyacrylamide gel electrophoresis (Figure 2). The material obtained from the resin migrated more slowly through the gel than unhybridized **9**, as expected for duplexed oligonucleotide.

In conclusion, we have demonstrated that a single photolabile solid phase support can be used to release protected oligonucleotides and/or as a platform for designing hybridization probes which have the novel feature that enables duplexed material to be released under mild nondenaturing conditions.

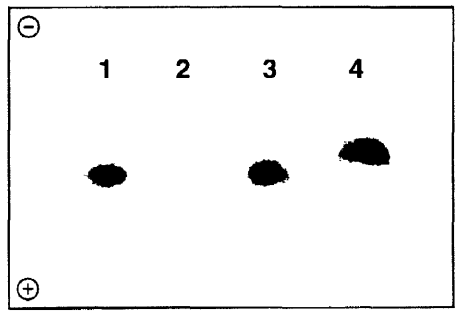


Figure 2. Nondenaturing (20%) polyacrylamide gel electrophoresis of hybridization studies involving **6**. Lane: 1, ^{32}P-**10**; 2, washings following photolysis of **6** hybridized with ^{32}P-**10**; 3, ^{32}P-**9**; 4, washings following photolysis of **6** hybridized with ^{32}P-**9**.

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12. Unlike LCAA-CPG, Tentagel® does not contain any base labile components. Also, Tentagel® has superior swelling properties relative to LCAA-CPG, which we anticipated would improve the hybridization efficiency of oligonucleotides prepared on the support.
13. Hybridizations were carried out at 45 °C in order to exploit the difference in melting temperatures of the respective duplexes between **6** and the complement (**9**, T_m = 52 °C) and the one base mismatch (**10**, T_m = 42 °C). Following heating of the resin containing **6** with the appropriate ^{32}P-labeled oligonucleotide in 100 μL phosphate buffer (10 mM, pH 7.5) containing NaCl (0.1 M) to 90 °C for 10 min, the mixture was allowed to cool in the heat block to 45 °C. The supernatants were removed via pipet, and the resin washed with phosphate buffer (3 × 50 μL). Photolyses were carried out in eppendorf tubes containing phosphate buffer using a transilluminator as described in reference 5. The supernatant was removed and analyzed by Cerenkov counting and/or nondenaturing polyacrylamide gel electrophoresis.