Dimethoxybenzoin Carbonates: Photochemically-Removable Alcohol Protecting Groups Suitable for Phosphoramidite-Based DNA Synthesis

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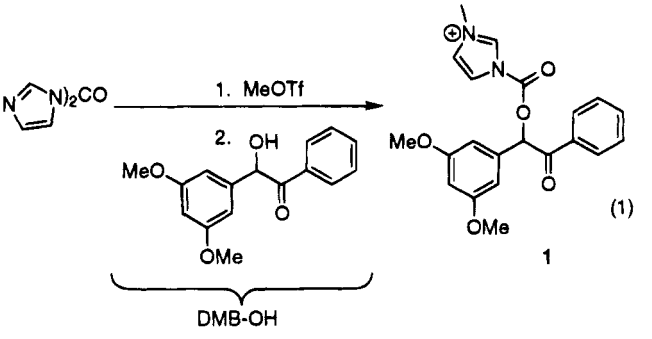
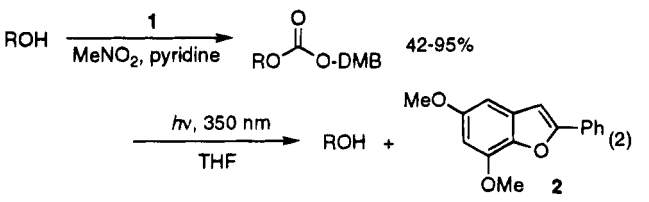
Photochemically-removable protecting groups[2] play an important part in a variety of technologies, including semiconductor lithography[3] and time-resolved studies in disciplines ranging from cell biology[4] to X-ray crystallography.[5] Nitrobenzyl derivatives[6] are most commonly used despite their drawbacks: a chemical step that follows the photochemistry can be rate limiting, and the nitrosoaldehyde byproduct is often reactive with sensitive functionalities and injurious to living systems. We have therefore developed some new photochemically-removable groups[7] that do not suffer these drawbacks, as have others.[8] We have exploited Sheehan's observations[9] of the reactivity of carboxylic esters of 3,5-dimethoxybenzoin (DMB) to develop protecting groups for phosphodiesters[10] and reasoned that conversion of DMB to its carbonate ester would convert it to a useful alcohol protecting group. The DMB-carbonate group can be used for the protection of the 5′-hydroxyl groups of nucleosides, permitting the development of a photochemical version of phosphoramidite-based DNA synthesis that should be useful for the application of light-directed spatially-addressable parallel synthesis[11] to the preparation of surface-bound arrays of DNA probes.[12]

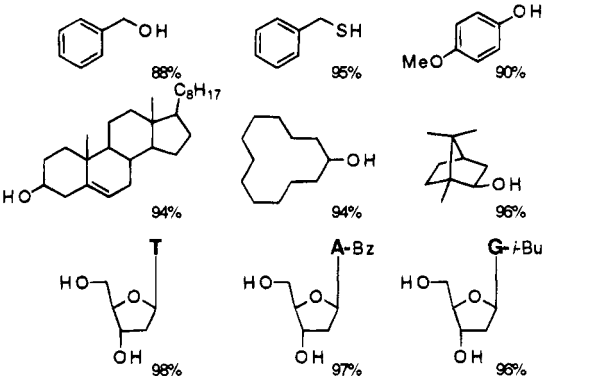
Activated carbonic acid derivatives such as chloroformates were initially examined for the derivatization of DMB to form an intermediate activated for the protection of alcohols. Invariably, these reactions led to cyclization to form an unsymmetrical bis-arylvinylene carbonate that was nucleophilically opened by the alcohol to provide a mixture of benzoin carbonates. However, carbonyldiimidazole activated by methylation[13] in nitromethane (0.5 M) proved uniquely capable of performing a single acylation of DMB (1 h, rt) to provide **1**, which was stable even at reflux temperatures (eq 1). Addition of a solution





of alcohol (0.5 equiv) followed by pyridine leads to acyl substitution to generate the DMB carbonate within 1 h at rt (eq 2). A selection of primary, secondary, and aryl alcohols and thiols was protected as shown in Table 1. In the case of the three nucleosides, high selectivity for the primary alcohol is observed. The DMB-carbonates are readily deprotected on irradiation with 350 nm lamps (Rayonet reactor) as dilute (<3 mM) solutions in benzene or acetonitrile. Typical reaction times are 1 h on a 0.25 mmol scale (2× longer in acetonitrile), and the isolated yields of the alcohols are uniformly high. Parallel irradiations of DMB acetate and benzyl DMB carbonate at low concentration show essentially the same rate of

Table 1. Alcohols Protected with DMB-carbonate and Yields in Their Deprotection in Benzene As Measured by Isolation of the Alcohol[a]



[a] Yields based on the benzofuran (BF) absorption were 80–92%. In one case (protected benzylmercaptan, BM), internal standard gas chromatography was used to measure the yields of both products, which were 102.8 ± 6.9% (BM) and 98.0 ± 6.9% (BF)

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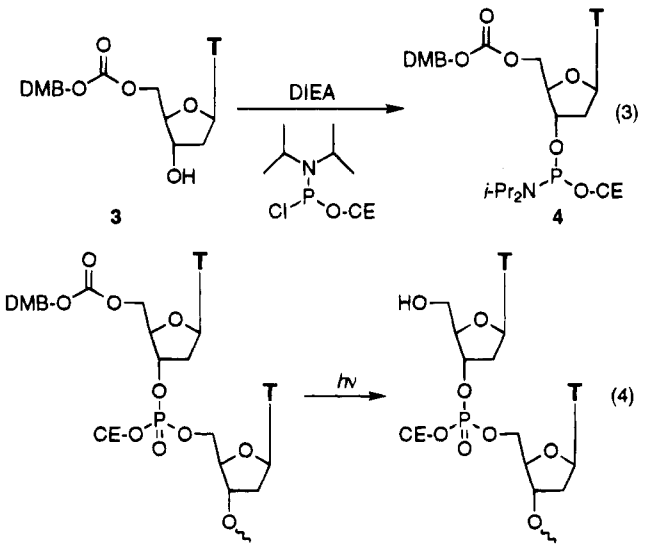
deprotection, suggesting the quantum yield of deprotection for these carbonates is similar to that reported for the acetate, 0.64. A significant advantage of these benzoin groups compared to nitrobenzyls is the inert phenyldimethoxybenzofuran byproduct. Its 300 nm absorbance and intense 396 nm fluorescence permit its detection at concentrations as low as 1 μM, permitting the yield of a photochemical deprotection to be directly measured by optical methods.

To demonstrate the utility of the DMB carbonate protecting group for the photochemical synthesis of DNA on a solid support, which is a prerequisite for the preparation of DNA probe arrays, two simple trinucleotides were prepared. Derivatization of T at the 5′ alcohol with the DMB-carbonate and conversion of the resulting **3** to the 3′-phosphoramidite **4** was accomplished by standard methods (eq 3).[14] Commercial long-chain



alkylamine controlled-pore glass (lcaa-cpg) beads derivatized with 5′-DMTr-T (25 μmol/g) were treated with acid and coupled to **4** under tetrazole catalysis with acetic anhydride/DMAP capping. Irradiation (eq 4) and coupling with 5′-DMTr-Bz-cytosine 3′-CE-phosphoramidite gave a trinucleotide. The protecting groups and tether were cleaved with ammonia and the product subjected to HPLC purification (reversed phase, 0.1 M Et_3N·HOAc/24–32% CH_3CN). The absorbance of the fraction containing the sequence 5′-DMTr-CTT was compared to that of a known concentration of an authentic sample (prepared by conventional DMTr synthesis) to determine an overall yield of 76.5%. The sequence 5′-ATT-3′ was prepared by first loading a lcaa-cpg support with 5′-DMB-CO_2-T (184 nmol by benzofuran release) through its 3′ succinate. Irradiation ($t_{1/2}$ = 2.7 min, total time = 30 min) and coupling with **4** was conducted as above. A second irradiation ($t_{1/2}$ = 3.3 min, total time = 45 min) and coupling (167 nmol by benzofuran release) with 5′-DMTr-Bz-adenosine 3′-CE-phosphoramidite gave a trinucleotide. It was cleaved from the support, analyzed by HPLC, and showed a single peak. This product was also purified using an OPC cartridge[15] and digested with snake venom phosphodiesterase/alkaline phosphatase.[16] The nucleosides in the resulting sample were analyzed by HPLC. A ratio of 64.5%:35.5% (T:A) was observed, consistent with the expected sequence. Despite the adjacent T residues in these two sequences, photochemical deprotection is not injurious to the DNA, as established by the absence of any T–T dimer product by comparison to an authentic sample.[17]

DMB-carbonates and the reagents developed in this work to generate them provide valuable new methods for light-controlled release of molecules.[18] In particular, they should offer much more rapid photochemical cleavage (<50 ns)[19] than nitrobenzyl protecting groups (ms).[20]

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Supplementary Material Available: Copies of ^1H and ^{13}C NMR spectra and experimental protocols for the protection and deprotection of alcohols and nucleosides (20 pages).

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