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A Universal Adapter for Chemical Synthesis of DNA or RNA on any Single Type of Solid Support

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Abstract: Use of a novel nucleotide adapter, 2'(3')-*O*-dimethoxytrityl-3'(2')-*O*-benzoyluridine-5'-*O*-(2-cyanoethyl *N,N*-diisopropylphosphoramidite) eliminates the need to have eight separate solid supports for synthesis of all possible oligoribo- and oligodeoxyribonucleotides.

Current strategies for chemical solid-phase synthesis of DNA and RNA oligonucleotides entail the use of eight supports, each bearing a separate nucleoside corresponding to the 3' terminus of the desired oligomer. As a way of simplifying this methodology, we originally introduced a single universal support that consisted of controlled pore glass[1] derivatized with 3-anisoyl-2'(3')-*O*-benzoyluridine 5'-*O*-succinyl residues and showed how it could be used to synthesize both DNA and RNA.[2] In a new and useful development employing this general concept, a fully protected nucleotide, 2'(3')-*O*-dimethoxytrityl-3'(2')-*O*-benzoyluridine-5'-*O*-(2-cyanoethyl *N,N*-diisopropylphosphoramidite), has now been synthesized and used in our laboratory as an all-purpose adapter, designed for synthesis of oligonucleotides on any of the commercially-available glass or polystyrene-based supports. Specifically, one variety of support, a polystyrene bearing thymidine residues, has been derivatized with the above phosphoramidite; subsequent removal of dimethoxytrityl from the now support-linked uridine adapter in a regular machine cycle exposes free ribonucleoside 2'(3') hydroxyl groups, onto which either DNA or RNA can be built. After the synthesis is complete, ammonia deblocking releases the chains from the support and cleaves the 3'-2'(3') phosphodiester linkages between the DNA or RNA molecules and the uridylyl-(5'-5')-thymidine moieties at their 3' ends, via 2',3'-cyclic phosphate formation on uridine.

The universal adapter, **7**, was prepared as shown (Figure 1). First, the intermediate 2', 3'-*O*-(dibutylstannylene)uridine (**2**), formed in quantitative yield from uridine, was converted to 3'-*O*-benzoyluridine (**3**) by the method of Wagner et al.[3] Protection of the 5'-hydroxyl of **3** with *t*-butyldimethylsilyl[4] was followed by tritylation of the remaining free secondary hydroxyl of the cis-diol[5] to form **5**. Subsequent removal of the silyl group[6] from **5** yielded the 5' hydroxyl compound **6** which was then phosphitylated[7] to give **7** (75% from **6**).

NMR analysis was conducted to confirm the structures of the adapter nucleotide and its synthetic precursors **3-6**. Proton NMR data[8] for **4** showed that migration of the benzoyl group between the 2'- and 3'-hydroxyls occurs under the conditions used for silylation of the 5'-hydroxyl. The ratio of areas under the H1' peaks[9] indicated an isomeric composition of 11% 2'-*O*-benzoyluridine and 89% 3'-*O*-benzoyluridine. ^{31}P NMR data confirmed the structure of **7**, and again demonstrated the presence of the two structural isomers, each with its corresponding R and S diastereomers.[8]

Derivatization of supports and subsequent oligonucleotide synthesis were carried out on a Gene Assembler Plus (Pharmacia) using the manufacturer's standard software protocols for DNA synthesis, without modification. Polystyrene solid support cassettes (Pharmacia) bearing 0.2, 1.3, and 10 μmole of

dimethoxytritylthymidine were successively mounted on the machine, detritylated, and derivatized with 2'(3')-*O*-dimethoxytrityl-3'(2')-*O*-benzoyluridine-5'-*O*-(2-cyanoethyl *N,N*-diisopropylphosphoramidite) delivered at a concentration of 0.15 M in acetonitrile. Coupling reaction time was 2 min. using 0.5 M tetrazole. Detritylations were carried out with 3% dichloroacetic acid in 1, 2-dichloroethane (v/v) for 1.3 and 10 µmole cassettes and 1% trichloroacetic acid in 1, 2-dichloroethane (v/v) for 0.2 µmole cassettes. Derivatized cassettes were flushed with acetonitrile and stored at -20° C until used for oligonucleotide synthesis.

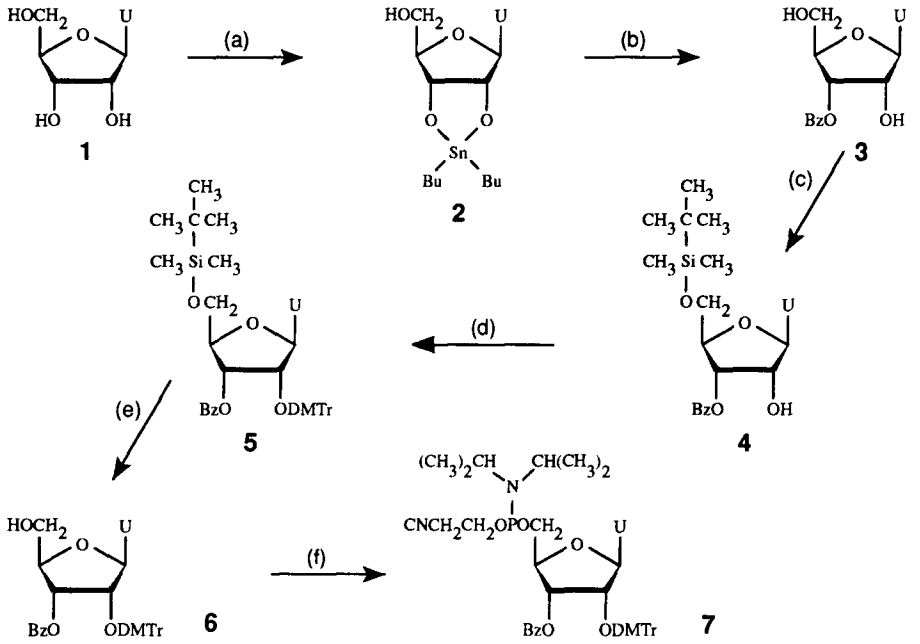

Fig. 1. Synthetic scheme for preparation of 2'(3')-*O*-dimethoxytrityl-3'(2')-*O*-benzoyluridine-5'-*O*-(2-cyanoethyl *N,N*-diisopropylphosphoramidite) (**7**). The reagents are (a) dibutyltin oxide; (b), benzoyl chloride; (c), *t*-butyldimethylsilyl chloride; (d) 4,4'-dimethoxytrityl chloride; (e) tetrabutylammonium fluoride; (f), 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite. For each of the compounds **3**, **4**, **5**, **6**, and **7**, the 3'-*O*-benzoyl isomer is shown.

The efficiency of the various couplings was measured by spectrophotometric estimation of dimethoxytrityl cation released by the acid treatment. Addition of **7** to the existing dT solid support involves coupling of a primary hydroxyl (5'-hydroxyl of support-bound dT) to the phosphite moiety attached to the 5' oxygen of the incoming adapter. This arrangement is most favorable sterically, and proceeds to essentially 100% yield. The addition of the first monomer to the adapter, however, requires coupling of the 3' phosphite moiety of the incoming 3' terminal nucleotide to the secondary 2'(3')-hydroxyl of the column bound adapter. Steric hindrance in this arrangement is compounded by the presence of the 2' protecting group of RNA synthetic units. Despite this unfavorable arrangement, coupling of the initial phosphoramidite, either RNA or DNA, proceeds to >98%. Moreover, multiple couplings of the adapter to itself using repeated cycles occur with a stepwise coupling efficiency of >99%.[8]

Oligonucleotides were synthesized using either β-cyanoethyl DNA phosphoramidites purchased from Pharmacia or RNA phosphoramidites synthesized as previously reported.[10] A variety of RNA and DNA molecules have been constructed using cassettes derivatized with the adapter, including the DNA oligomer dC-dG-dA-dA-dG-dT-dG and the RNA oligomer G-C-U-G-U-C-A-C-C-G-C-G. Anion exchange HPLC profiles for these syntheses (Figure 2) show the components present in the crude deprotection[11] mixtures.

Used in the manner outlined above, the adapter permits oligonucleotides to be constructed on any type of support and therefore represents a considerable improvement in the simplicity and economy of solid phase oligonucleotide synthesis.

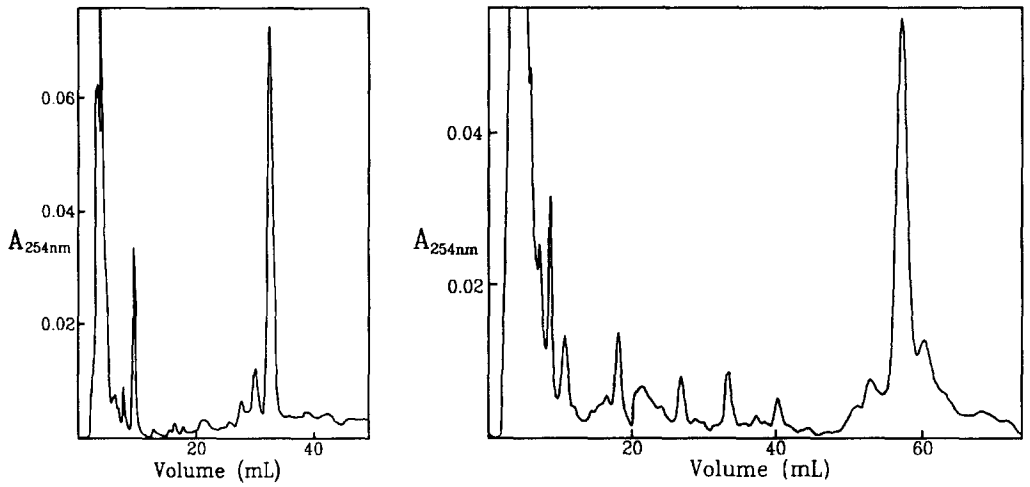

Fig. 2. HPLC elution profiles of DNA oligomer dC-dG-dA-dA-dG-dT-dG (left) and RNA oligomer G-C-U-G-U-C-A-C-C-G-C-G (right) which were constructed using the universal adapter. A crosslinked polyethyleneimine-silica column[12] (0.4 X 25 cm) was used for analysis, and elution was carried out with 150 mL and 100 mL, respectively, of the solvent system: aqueous 0.05 M KH_2PO_4 containing 30% methanol with a linear gradient of 0-0.5 M $(NH_4)_2SO_4$ at pH 6.0 (flow rate 1 mL/min).

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References and Notes

1. Gough, G. R.; Brunden, M. J.; Gilham, P. T. *Tetrahedron Letters* **1983**, *24*, 5321-5324.

2. deBear, J. S.; Hayes, J. A.; Koleck, M. P.; Gough, G. R. *Nucleosides Nucleotides* **1987**, *6*, 821-830.

3. Wagner, D.; Verheyden, J. P. H.; Moffatt, J. G. *J. Org. Chem.* **1974**, *39*, 24-30.

4. Ogilvie, K. K.; Beaucage, S. L.; Schifman, A. L.; Theriault, N. Y.; Sadana , K. L. *Can. J. Chem.* **1978**, *56*, 2768-2780.

5. Compound **5** was synthesized as follows: 5'-*O*-(*t*-butyldimethylsilyl)-3'(2')-*O*-benzoyluridine (1.60 g, 3.45 mmol) was dried by coevaporation with anhydrous pyridine (3 X 20 mL). The nucleoside was dissolved in 4.5 mL dry pyridine, and treated with dimethoxytrityl chloride (1.37 g, 4.05 mmol). After 24 h at 60° C, the solution was concentrated to an oil, which was dissolved in ethyl acetate (150 mL). The organic layer was extracted with 5% $NaHCO_3$ (3 X 100 mL), washed with 10% NaCl (3 X 100 mL), dried with anhydrous Na_2SO_4, and evaporated to a foam. This material was purified on a 2.5 X 30 cm column of silica gel 60 eluted with solvent mixtures containing 0.25% pyridine (v/v): 200 mL chloroform, then 200 mL each of 0.25%, 0.50%, 0.75%, and 1% of methanol in chloroform (v/v). Final purification was carried out by high-resolution centrifugal chromatography using 400 mL of 1% methanol : chloroform (v/v) with 0.25% pyridine. Compound **5** was obtained in 83% yield.

6. For compound **6**: 5'-*O*-(*t*-Butyldimethylsilyl)-2'(3')-*O*-dimethoxytrityl-3'(2')-*O*-benzoyluridine (1.83 g, 2.39 mmol) was treated with tetrabutylammonium fluoride (3.41 mL of a 1 M solution in THF). After 6 h stirring at room temperature, the reaction mixture was diluted with ethyl acetate (150 mL), extracted with 5% $NaHCO_3$ (3 X 100 mL) , washed with 10% NaCl (3 X 100 mL), and dried with anhydrous Na_2SO_4. The organic layer was evaporated to a slurry, and this material was purified on a 2.5 X 30 cm column of silica gel 60 using a stepwise gradient with 0.25% pyridine in the eluant. The gradient was as follows: 200 mL chloroform, 200 mL each of 0.5%, 1%, 1.5% and 2% methanol in chloroform (v/v). The final purification was carried out by centrifugal thin layer chromatography using 400 mL of 2.5% methanol : chloroform (v/v) containing 0.25% pyridine. The yield of **6** was 98%.

7. Sinha, N. D.; Biernat, J.; McManus, J.; Koster, H. *Nucleic Acids Res.* **1984**, *12*, 4539-4557.

8. Schwartz, M. E., Ph. D. Thesis; Purdue University, 1992.

9. Fromageot, H. P. M.; Griffin, B. E.; Reese, C. B.; Sulston, J. E.; Trentham, D. R. *Tetrahedron* **1966**, *22*, 705-710.

10. Schwartz, M. E.; Breaker, R. R.; Asteriadis, G. T.; deBear, J. S.; Gough, G. R. *BioMed. Chem. Lett.* **1992**, *2*, 1019-1024.

11. Deprotection of the detritylated oligomers was carried out on free resin removed from the manufacturer's cassettes. The deoxyribo-oligomer was deprotected[2] in a pressure vial with 25 mL concentrated NH_4OH for 48 h at 65° C. The mixture was then filtered and concentrated under reduced pressure. The ribo-oligomer was deprotected with 25 mL pyridine:conc. NH_4OH (1:4, v/v) for 24 h at 50° C. The mixture was filtered and co-evaporated with *t*-butanol:water (1:1, v/v), then irradiated with long-wave UV light at pH 3.7 for 4.5 h. at 37° C to remove its 2'-*O*-(*o*-nitrobenzyloxymethyl) protecting groups.[10]

12. Lawson, T. G.; Regnier, F. E.; Weith, H. L. *Anal. Biochem.* **1983**, *133*, 85-93.

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