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Facile preparation of 3´-derivatized oligodeoxynucleotides

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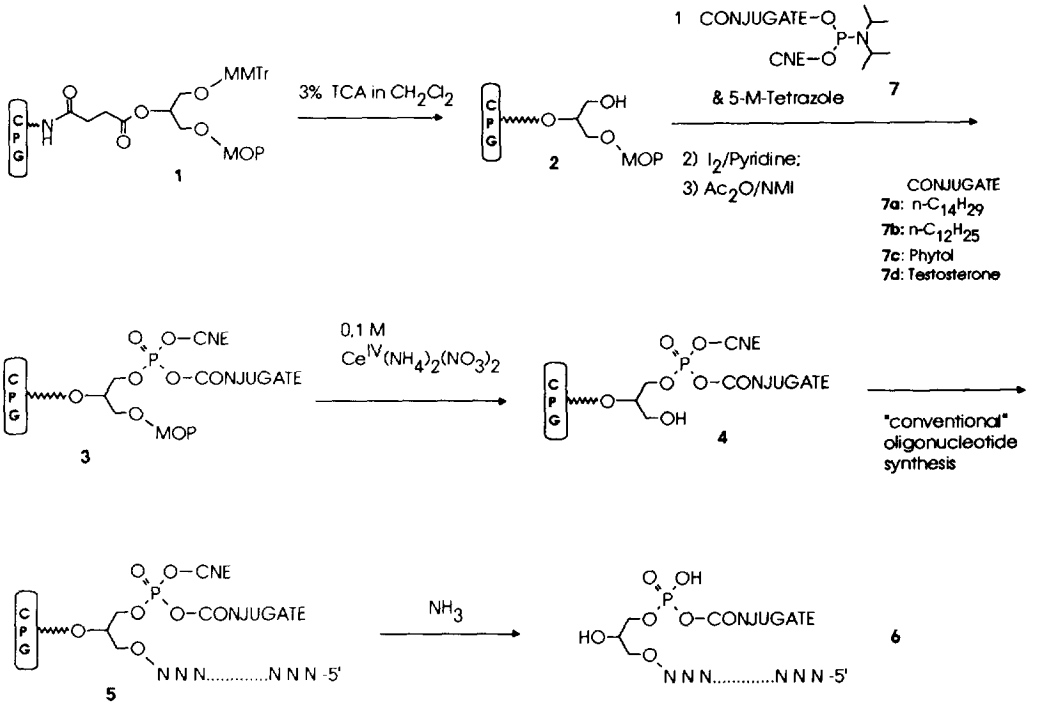
Abstract: We present a facile method for the preparation of 3'-derivatized oligonucleotides. It involves the use of a universal solid support coupled linker molecule 1 carrying two orthogonally protected hydroxyl groups: the 4-methoxytriphenylmethylether and the 4-methoxyphenylether. 1 allows the introduction of 3'-conjugate molecules into oligonucleotides during solid phase synthesis with the advantage that the same reagents can be used as for 5'-modification.

The attachment of conjugate molecules to the 3'- or 5'-terminus of synthetic oligonucleotides has improved their diagnostic utility as DNA probes[1,2] and their potential therapeutic applications in antisense oligonucleotides.[3-5] Digestion of oligonucleotides by 3'-exonucleases, which is the main pathway of oligonucleotide degradation, can be considerably reduced by 3'-conjugates, making them a particularly important modification. 5´-terminal modifications are easily introduced during solid phase synthesis,[4-6] provided that the group which has to be linked can be derivatized as a phosphoramidite, H-phosphonate or phosphodiester and is stable under the coupling and deprotection conditions. No such universal method exists for 3'-terminal modifications since the oligonucleotide is coupled to the solid support at its 3'-end during solid phase synthesis. Methods for 3'-terminal derivatization include post-synthetic labeling of 3'-amino or 3'-thiol-containing oligonucleotides[7,8] or the preparation of solid supports that contain the conjugate molecule as a first building block instead of the first nucleoside monomer of the oligonucleotide.[9] Both methods are laborious and less universally applicable as compared to 5'-terminal modification.

Here we present an easy method for the 3'-derivatization of oligonucleotides during solid phase synthesis which is outlined in *Scheme 1*. The method is based on a new linker molecule 1 carrying two orthogonally protected hydroxyl groups: the 4-methoxytriphenylmethylether (MMTr) and the 4-methoxyphenylether (MOP). First, the MMTr group of 1 is cleaved by treatment with 3% trichloroacetic acid in dichloromethane. The resulting free hydroxyl group in 2 is reacted with the conjugate containing reagent, preferably using conventional phosphoramidite derivatives such as compound 7. Best results were obtained using 5-methylthio-1H-tetrazole as activator in the coupling reaction. One major advantage of this 3'-derivatization method is that the same reagents can be used as for 5'-modification. It is mandatory that the second protecting group must survive the

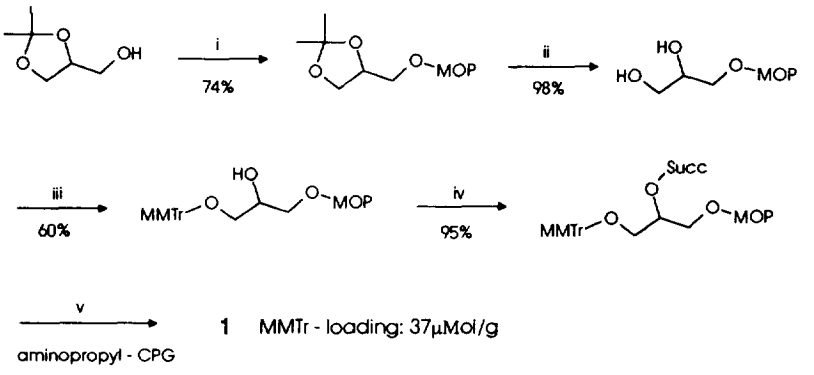
phosphoramidite coupling cycle: MMTr-deprotection, coupling, oxidation with iodine/pyridine and capping with acetic anhydride/N-methylimidazole. Moreover, the cleavage conditions for this group should not interfere with the phosphate protecting group, usually a 2-cyanoethylester (CNE). The chosen MOP group fulfills these conditions since it is stable to acidic and basic conditions and also resistant to iodine oxidation. Its removal by oxidative cleavage with $Ce^{IV}(NH_4)_2(NO_3)_2$ leaves the cyanoethylphosphate group of 3 unchanged. The single free hydroxyl group in 4 now serves as the starting point for conventional oligonucleotide synthesis. Deprotection and cleavage from CPG using concentrated ammonia yields the 3'-conjugated oligonucleotides 6. The method is of course limited to conjugates which are not oxidized by Ce^{IV}.



Scheme 1: 3'-derivatization of oligonucleotides. TCA: trichloroacetic acid; NMI:N-methylimidazole; Ac_2O: acetic anhydride; CNE: 2-cyanoethyl; MMTr: 4-methoxytriphenylmethyl; MOP: 4-methoxyphenyl; CPG: Controlled Pore Glass. 5-M-tetrazole: 0.2M solution of 5-methylthio-1H-tetrazole in CH_3CN. Oxidation with Ce^{IV} is carried out using a 0.1 M solution of $Ce^{IV}(NH_4)_2(NO_3)_2$ in CH_3CN/H_2O (4:1 v/v), 25°C, 5 min. Oligonucleotide synthesis is carried out following the standard sythesis protocol for the ABI 394 DNA synthesizer.

The preparation of the CPG-coupled linker molecule 1 is outlined in *Scheme 2*. 2,2-Dimethyl-4-hydroxymethyl-1,3-dioxolan is reacted with 4-methoxyphenol in a Mitsunobu reaction. Subsequently, the acetal is cleaved with acetic acid, followed by the introduction of the MMTr and succinic acid group. Finally, the linker molecule is

coupled to aminopropyl CPG or other suitable polymeric supports. After capping with acetic anhydride , the monomethoxytrityl loading was determined to be 37 μMol/g.



Scheme 2: synthesis of linker molecule 1: i) 3 eq. 4-methoxyphenol, 1.3 eq. triphenylphosphine, 1.3 eq. diethylazodicarboxylate, THF, reflux, 1h; ii) 80% CH_3COOH, 25°C, 4h; iii) 1.1 eq. MMTr-Cl, pyridine, 25°C, 3h; iv) 1.4 eq. succinic anhydride, 1.4 eq. N,N-dimethylaminopyridine, pyridine, 19h; v) 1.1 eq. DCC, 1 eq. 4-nitrophenol,0.2 eq. aminopropyl-CPG (Fluka, 0.1 mmol/g, 550 Å), pyridine, 25°C, 7h.

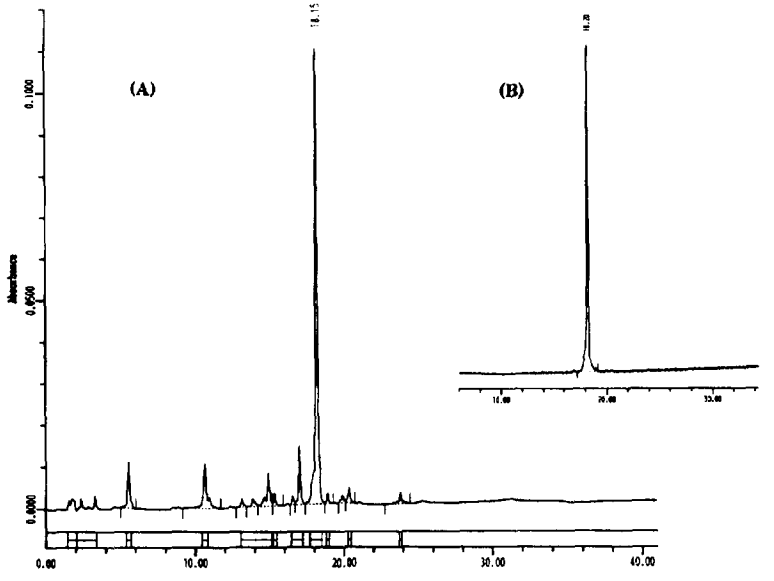


Figure 1: HPLC traces of crude (A) and purified (B) 3'-conjugated oligonucleotide 5'-TpTpTpTpTpTpTp-O-CH_2-CH(OH)-CH_2-p-O-$C_{14}H_{29}$

To test the utility of the conjugation procedure, the sequence dT_8, was conjugated at the 3'-end with tetradecane-1-O-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite 7a on a 0.5 μMol scale. Coupling of 7a with 2 was carried out two times following the standard protocol on an ABI 394 DNA synthesizer. After capping with acetic anhydride/N-methylimidazole, the MOP group was cleaved using a 0.1M $Ce^{IV}(NH_4)_2(NO_3)_2$ solution in acetonitrile/water (4:1) at 25°C for 5 min. After extensive washing with acetonitrile the oligonucleotide was assembled following the ABI standard protocol. Coupling efficiencies, determined by measurement of the MMTr cation released during deprotection, were > 97%. Cleavage from the solid support and deprotection were carried out with conc. aqueous ammonia at 55°C for 12 hr. The ammonia solution was concentrated and the oligonucleotide (the yield of crude material was 18 OD) was purified by HPLC and desalted by gel filtration over Sephadex (NAP-G25 column, Pharmacia). Figure 1 shows the ion exchange HPLC trace of the crude and the purified oligonucleotide (Waters GenPak FAX, gradient CH_3CN/H_2O (1:4 (v/v), 0.01M NaH_2PO_4, 0.1M NaCl to CH_3CN/H_2O (1:4 (v/v), 0.01M NaH_2PO_4, 1.5M NaCl). The purity and integrity of the conjugated oligonucleotide was confirmed by negative electrospray mass spectroscopy (Fisons Bio-Q; calculated: 2802.0; found: 2823.81 [M+Na]). Other molecules that were coupled to the 3'-end of oligonucleotides include n-$C_{12}H_{25}$, phytol and testosterone.

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References:

1. Tenover, F. C. *Clin. Microbiol. Rev.* **1988**, *1*, 82-101.
2. Englisch, U; Gauss, D. *Angew. Chem.* **1991**, *103*, 629-646.
3. Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543-584.
4. Manoharan, M.: Designer Antisense Oligonucleotides: Conjugation Chemistry and Functio-nality Placement. In *Antisense Research and Applications*; Crooke, S.; Lebleu, Eds.; CRC Press, Boca Raton 1993; pp. 303-349.
5. Goodchild, J. *Bioconj. Chem.* **1990**, *1*, 165-187.
6. Sinha, N. D.; Striepeke: Oligonucleotides with reporter groups attached to the 5'-terminus. In *Oligonucleotides and Analogues - A Practical Approach*; Eckstein, F., Ed., Oxford University Press, New York 1991, pp185-210.
7. Agrawal, S.: Functionalization of Oligonucleotides with Amino Groups and Attachment of Amino Specific Reporter Groups. In *Protocols for Oligonucleotide Conjugates*; Agrawal, S., Ed.; Methods in Molecular Biology, Vol. 26, Humana Press, Totowa 1994, pp.93-120.
8. Nelson, P.S., Kent, M., Muthini, S. *Nucleic Acids Res.* **1992**, *20*, 6253-6259.
9. Gamper, H. B.; Reed, M. W.; Cox, T.; Virosco, J. S.; Adams, A. D.; Gall, A. A.; Scholler, J. K.; Meyer Jr., R. B. *Nucleic Acids Res.* **1993**, *21*, 145-150.

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