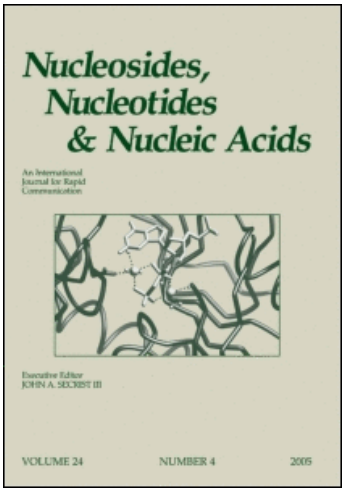

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Jari Hovinen[a]
[a] PerkinElmer Life and Analytical Sciences, Turku Site, Turku, Finland

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LABELING OF OLIGONUCLEOTIDES WITH DTPA AND DOTA ON SOLID PHASE

Jari Hovinen □ *PerkinElmer Life and Analytical Sciences, Turku Site, Turku, Finland*

□ *Oligonucleotide conjugates labeled with metal chelates of diethylenetriaminepentaacetic acid (DTPA) and tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) were synthesized on solid phase using appropriate nucleosidic phosphoramidite building blocks (**3**, **4**) and a modified deprotection-metal chelation protocol. The major differences on the properties of the oligonucleotide conjugates also are discussed.*

Keywords Oligonucleotide; DTPA; DOTA

Because of their excellent metal chelating properties 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA, **1**) and diethylenetriaminepentaacetic acid (DTPA, **2**; Chart 1) are widely used as organic ligands in magnetic resonance imaging (MRI), positron emission tomography (PET) and single photon emission computed tomography (SPECT). Tethered to bioactive molecules, they can find applications even as target-specific radiopharmaceuticals.[1]

In several applications, covalent conjugation of **1** or **2** to oligonucleotides is required. Several bifunctional DTPA and DOTA derivatives which allow oligonucleotide conjugation are even commercially available. However, since the labeling reaction is performed in solution in the presence of an excess of an activated chelate, laborious purification procedures cannot be prevented. Especially, when attachment of several label molecules is needed, purification and characterization of the desired oligonucleotide conjugate may be extremely difficult. These problems can be avoided by performing the labeling reaction on solid phase using DTPA and DOTA derivatives (**3**,**4**; Chart 1) and standard machine assisted phosphoramidite chemistry.[2,3]

The blocks (**3**, **4**) can be coupled to the oligonucleotides using 10 minutes coupling time and 0.2 M concentration with excellent

Address correspondence to Jari Hovinen, PerkinElmer Life and Analytical Sciences, Turku Site, P.O. Box 10, FIN-20101 Turku, Finland. E-mail: jari.hovinen@perkinelmer.com

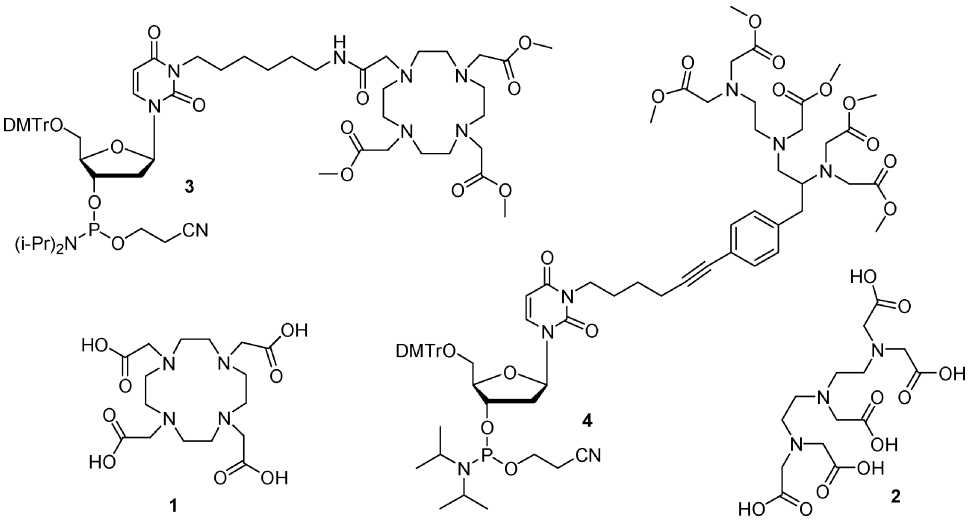

CHART 1

coupling efficiency. Upon completion of the syntheses (DMTr-Off), the oligonucleotide conjugates are deprotected and converted into the appropriate metal chelates in the following way: (i) treatment with 0.1 M NaOH for 4 hours at rt, (ii) concentration in vacuo in the presence of ammonium chloride; (iii) treatment with conc. aqueous ammonia for 16 hours at 55°C; (iv) treatment with the appropriate metal salt (e.g.; gadolinium(III) citrate); (v) desalting by gel filtration (NAP 5); (vi) denaturing 20% PAGE; (vii) passive elution from the gel to aqueous solution (Na_2CO_3 buffer, pH 9.8); (viii) butanol concentration; (ix) desalting by gel filtration (NAP 5). In the case of DOTA a prolonged reaction time (overnight at rt) and a 15-fold excess of metal salt is required to ensure complete chelate formation, while in the case of DTPA the chelation is completed in 2 hours using 5 equivalents of the metal per ligand.

The major advantages of the present method are: (i) The blocks can be introduced to the oligonucleotide structure with a standard oligonucleotide synthesizer in high efficiency using normal procedures; (ii) the method allows multilabeling (this is very advantageous in applications where high detection sensitivity is required) (iii) since the metal is introduced after the chain assembly is completed, the molecule synthesized can be used in various applications simply by changing the metal; (iv) because of the synthetic strategy the oligonucleotide conjugate is always free from unconjugated chelate (this is extremely important in vivo applications). It is worth noting that the labels attached at the *N*3 position of uracil residues naturally weaken hydrogen bonds in the duplex. Thus, these labels should be used only up or downstream of the coding sequence.

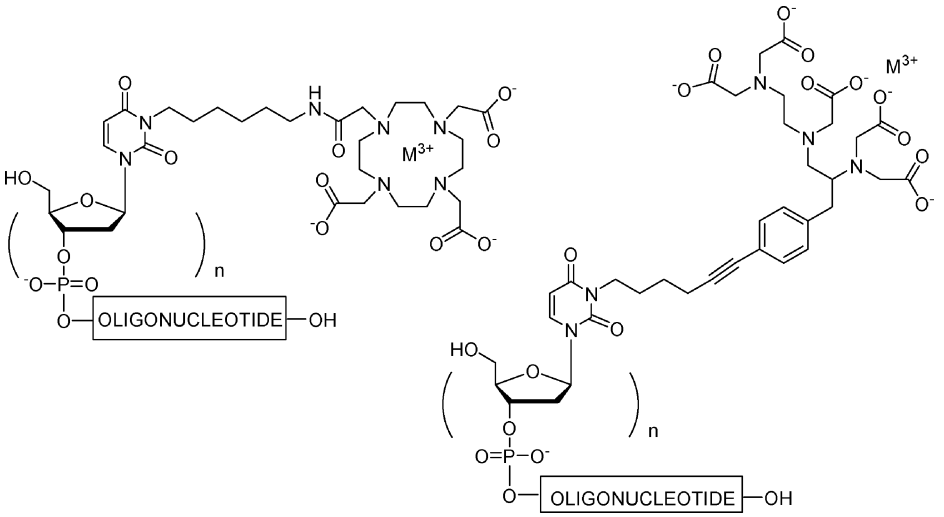

CHART 2 Structures of DOTA (1), DTPA (2), and the corresponding oligonucleotide labeling reactants.

The major differences of oligonucleotides labeled with DTPA and DOTA. It is known that the negatively charged Gd-DTPA distributes throughout the extracellular and intravascular fluid spaces, but does not cross an intact blood-brain barrier. Accordingly, oligonucleotides labeled with DTPA have lower cell permeability than the corresponding intact molecules (Chart 2). This diminishes the suitability of DTPA chelates to in vivo applications. Furthermore, it has been reported that the in vivo stability of DTPA is not always high enough.[4] This may be a serious problem when highly toxic metal ions have to be used.

The above mentioned problems can be avoided using oligonucleotides labeled with neutral and more stable DOTA derivatives. However, because of its slow kinetics of chelate formation, the use of DOTA is problematic in applications where short-living radioisotopes, such as ^{68}Ga are required. However, the chelate formation may be accelerated using microwave radiation.[5] In DELFIA assays, in turn, where the chelate has to be rapidly dissociated in acidic conditions,[6] the lanthanide(III) DOTA chelates are too stable, and the use of chelates based on DTPA is recommended.

As a summary, a straightforward method for the preparation of oligonucleotides labeled with DTPA and DOTA is presented. The selection of the ligand is strongly dependent on the application.

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