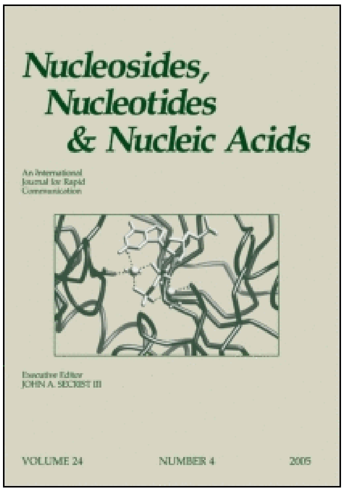

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PERYLENE DIIMIDE–OLIGONUCLEOTIDE CONJUGATES CONSTRUCTED BY CLICK CHEMISTRY

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□ *Oligonucleotide conjugates were prepared by copper-catalyzed cycloaddition of acetylene-modified oligonucleotides to diazido derivative of perylene 3,4,9,10-tetracarboxylic acid diimide.*

Keywords Oligonucleotides; conjugates; click chemistry; perylene diimide; [3+2] cycloaddition

The copper-catalyzed cycloaddition reaction of azides to alkynes[1,2] (Scheme 1) is a remarkable representative of so-called "click-chemistry"[3] reactions that are useful for bioconjugate synthesis. The process is being carried out regioselectively in mild conditions, tolerated by a majority of functional groups, affords great opportunities for the preparation of modified oligonucleotides,[4,5] and oligonucleotide immobilization.[6]

Conjugates of oligonucleotides with photostable perylene diimide dyes were extensively studied because of their interesting ability to self-assembly.[7–9] The perylene diimide moiety located in the middle of an oligonucleotide strand is known to increase its propensity to triplex formation.[10,11]

Although phosphoramidite reagents[9,10] were used to introduce perylene diimide into the strand, the preparation of conjugates with two strands of the same polarity flanking a single dye is not straightforward. Herein we would like to report a click chemistry approach to these products.

An introduction of reactive group into an oligonucleotide is required for conjugation to take place. Since the azido group is incompatible with

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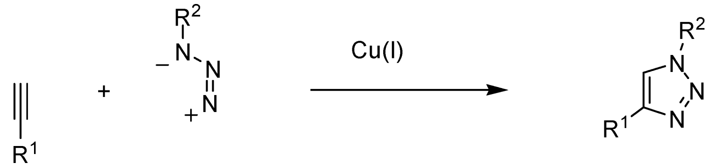
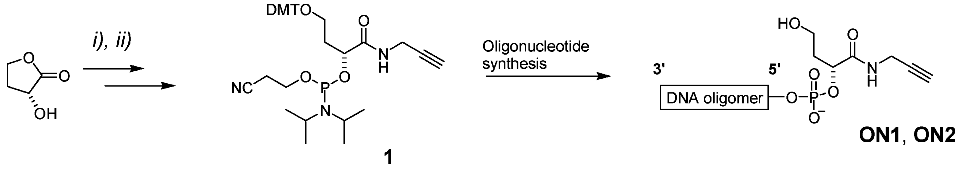


SCHEME 1 Copper-catalyzed cycloaddition of azides to alkynes.



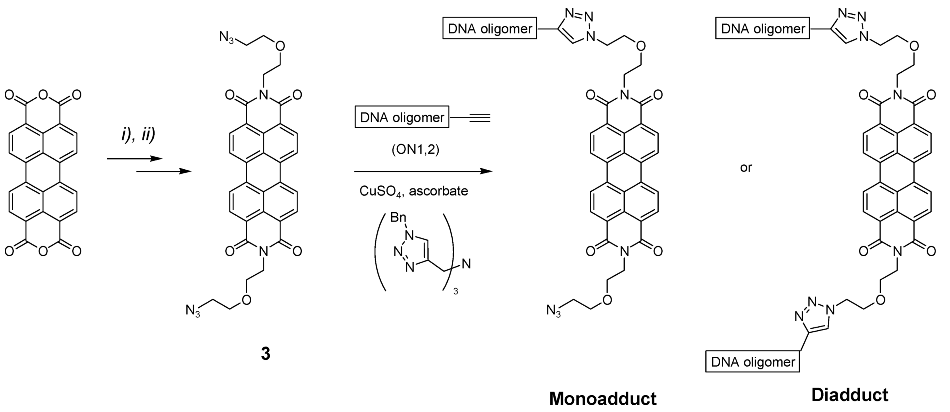
Reagents and conditions: *i)* Propargylamine, MeOH, 60°C, 48 h; 4,4′-dimethoxytrityl chloride, Py, 0°C to rt, 12 h, 86%; *ii)* 2-cyanoethoxy-bisdiisopropylaminophosphine, diisopropylammonium tetrazolide, rt, 12 h, 93%

SCHEME 2 Synthesis of acetylenic oligonucleotides

phosphoramidite synthesis because of Staudinger reaction,[12] we decided to introduce a terminal alkyne using a dihydroxybutyramide[13] reagent **1** prepared from propargylamine and (*R*)-2-hydroxybutyrolactone (Scheme 2).

Oligonucleotides (Table 1) were prepared from reagent **1** and solid support **2** by means of standard oligonucleotide synthesis.

Perylene diimide-derived diazide **3** prepared from perylenetetracarboxylic acid dianhydride in two steps (Scheme 3) was then coupled to oligonucleotides to give corresponding conjugates (Table 2). The reaction



Reagents and conditions: *i)* 2-(2-hydroxyethoxy)ethylamine, 130°C, 3 h, 94%; *ii)* $(PhO)_2PON_3$, Ph_3P, DEAD, THF, rt, 12 h, 98%.

SCHEME 3 Synthesis of perylene diimide-oligonucleotide conjugates.

TABLE 1 Oligonucleotides containing terminal alkyne (**X** = residue of **1**)

#	Sequence, 5′ → 3′
ON1	**X**CA-TTA-CAT-CCA-GAC
ON2	**X**GT-CTG-GAT-GTA-ATG

TABLE 2 Yields of conjugates of acetylenic oligonucleotides with diazide **3**

		Products[a]			
		Monoadduct		Diadduct	
Starting oligonucleotide	Oligonucleotide/azide	Yield	MALDI[b]	Yield	MALDI[b]
ON1	1:1	36%	5029.8/5028.5	42%	9444.7/9439.4
ON1	2:1	—		41%	
ON2	1:1	35%	5171.1/5170.6	35%	9729.2/9723.6
ON2	2:1	—		59%	

[a] Isolated yield (PAGE), determined by UV absorbance

[b] MALDI-TOF mass (M+H), found/calculated

was carried out in aq. DMSO under argon. Tris[(1-benzyltriazol-4-yl)methyl]amine[14] was used as a ligand for Cu(I) species to decrease copper oxidation accompanied by free hydroxyl radical (·OH) formation leading to oligonucleotide cleavage. Heating (95°C to rt, overnight) was required to facilitate dissolution of azide and increase overall yield of conjugation.

The conjugates can be isolated either by PAGE or by HPLC. It is possible to prepare diadducts of oligonucleotides **ON1** and **ON2** selectively using 2 equiv. of oligonucleotide.

We are planning to study the fluorescent properties of these conjugates as well as their use for assembly of DNA-nanostructures.[15]

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