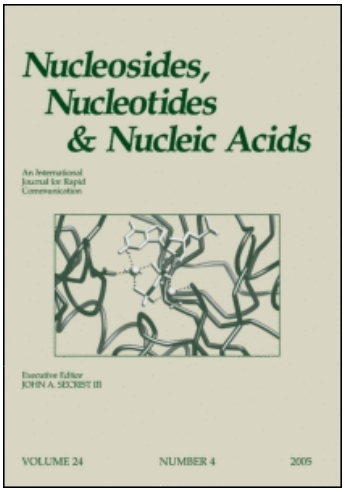

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Synthesis of Base-Modified Oligonucleotides Containing 6- and 7-Aryl Lumazines

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CHEMISTRY

SYNTHESIS OF BASE-MODIFIED OLIGONUCLEOTIDES CONTAINING 6- AND 7-ARYL LUMAZINES

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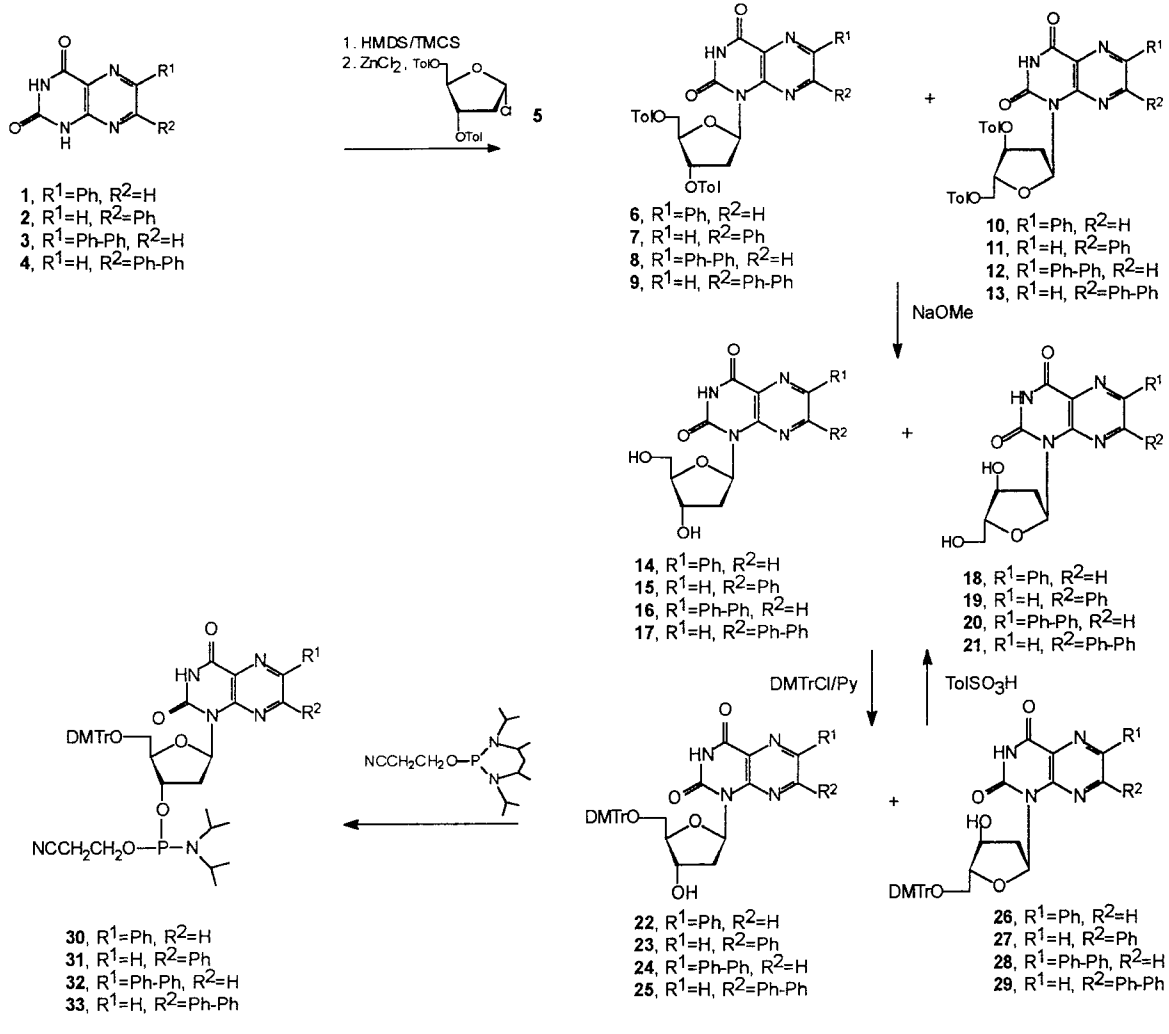
Abstract. 6-Phenyl, 7-phenyl, 6-(4-biphenyl)-, 7-(4-biphenyl)lumazine N^1-(2'-deoxy-D-ribofuranosides) were synthesized and incorporated in the different positions of self-complementary oligodeoxyribonucleotides, and the influence of modifications on the melting points of duplexes was studied.

Lumazine N^1-(2'-deoxy-D-ribofuranosides), which can be regarded as structural analogs of thymidine, bearing substituents both at 6- and 7-position of the lumazine moiety or unsubstituted, were synthesized and incorporated into oligonucleotides,[1-4] to study the influence of lumazine moiety on hybridization and fluorescence properties of oligonucleotides. Now we are reporting the synthesis of monosubstituted 6- or 7-phenyl and 6- or 7-(biphenyl)lumazine N^1-(2'-deoxy-D-ribofuranosides).

Synthesis of nucleotides **6-13** was accomplished by the reaction of lumazine derivatives **1-4** with 2-deoxy-3,5-di-O-p-toluoyl-D-erythropentofuranosyl chloride **5** (Scheme). Lumazines **1-4** (1.4 eq.) were silylated with hexamethyldisilazane and trimethylchlorosilane in acetonitrile, $ZnCl_2$ (0.5 eq.), and a solution of **5** (1.0 eq.) in CH_2Cl_2 during 3 h at -25°C was dropped into the reaction mixture. An anomeric mixture of nucleosides **6-13** was obtained after flash chromatography. Increasing the reaction temperature, amount of $ZnCl_2$, sugar **5**, decreasing the addition time of **5**, or changing the solvents (chloroform, 1,2-dichloroethane) led to the increasing amounts of α-anomers **10-13** or to the mixtures of N^1,N^3-diribosides (up to 30%).

Separation of the anomers by the recrystallization from chloroform-acetone was successful only in the case of 7-substituted lumazines which allowed to obtain ß-anomers **7** (39%) and **9** (58%) and α-anomers **11** (16%) and **13** (19%), which were deacylated with NaOMe in MeOH to give the deprotected nucleosides **15**, **17**, **19** and **21** (yield 86-93%). Inseparable anomeric mixtures (both by recrystallization and chromatography) of 6-substituted ribosides **6/10** and **8/12** were deprotected with NaOMe in MeOH, with treatment by DMTrCl to obtain 5'-dimethoxytrityl derivatives **22/26** and **24/28**, which could be easily separated by flash chromatography. Detritylation with 1% p-toluenesulphonic acid in CH_2Cl_2/MeOH (4:1) gave the individual nucleosides **14**, **16**, **18** and **20** (yield 66-89%). Treatment of **15**, **17**, **19** and **21** with DMTrCl in pyridine gave 5'-dimethoxytrityl derivatives **23**, **25**, **27** and **29**.



1, R^1=Ph, R^2=H
2, R^1=H, R^2=Ph
3, R^1=Ph-Ph, R^2=H
4, R^1=H, R^2=Ph-Ph

6, R^1=Ph, R^2=H
7, R^1=H, R^2=Ph
8, R^1=Ph-Ph, R^2=H
9, R^1=H, R^2=Ph-Ph

10, R^1=Ph, R^2=H
11, R^1=H, R^2=Ph
12, R^1=Ph-Ph, R^2=H
13, R^1=H, R^2=Ph-Ph

14, R^1=Ph, R^2=H
15, R^1=H, R^2=Ph
16, R^1=Ph-Ph, R^2=H
17, R^1=H, R^2=Ph-Ph

18, R^1=Ph, R^2=H
19, R^1=H, R^2=Ph
20, R^1=Ph-Ph, R^2=H
21, R^1=H, R^2=Ph-Ph

22, R^1=Ph, R^2=H
23, R^1=H, R^2=Ph
24, R^1=Ph-Ph, R^2=H
25, R^1=H, R^2=Ph-Ph

26, R^1=Ph, R^2=H
27, R^1=H, R^2=Ph
28, R^1=Ph-Ph, R^2=H
29, R^1=H, R^2=Ph-Ph

30, R^1=Ph, R^2=H
31, R^1=H, R^2=Ph
32, R^1=Ph-Ph, R^2=H
33, R^1=H, R^2=Ph-Ph

TABLE

The list of prepared oligonucleotides and their T_m values[a]

No.	Oligonucleotide sequence	Lu	T_m (°C)
	5'-d(GG-TT-CC-AT-GC-AT-GG-AA-CC)	-	60.4
1.	5'-d(GG-TT-CC-AT-GC-A**Lu**-GG-AA-CC)	6-Ph	60.0
2.	5'-d(GG-TT-CC-A**Lu**-GC-A**Lu**-GG-AA-CC)	6-Ph	65.5
3.	5'-**Lu**-d(GG-TT-CC-AT-GC-AT-GG-AA-CC)	6-Ph	60.8
4.	5'-d(GG-TT-CC-AT-GC-A**Lu**-GG-AA-CC)	7-Ph	61.5
5.	5'-d(GG-TT-CC-A**Lu**-GC-A**Lu**-GG-AA-CC)	7-Lu	63.4
6.	5'-**Lu**-d(GG-TT-CC-AT-GC-AT-GG-AA-CC)	7-Lu	60.9
7.	5'-d(GG-TT-CC-AT-GC-A**Lu**-GG-AA-CC)	6-Ph-Ph	57.3
8.	5'-d(GG-TT-CC-A**Lu**-GC-A**Lu**-GG-AA-CC)	6-Ph-Ph	-[b]
9.	5'-**Lu**-d(GG-TT-CC-AT-GC-AT-GG-AA-CC)	6-Ph-Ph	59.4
10.	5'-d(GG-TT-CC-AT-GC-A**Lu**-GG-AA-CC)	7-Ph-Ph	64.3
11.	5'-d(GG-TT-CC-A**Lu**-GC-A**Lu**-GG-AA-CC)	7-Ph-Ph	70.2
12.	5'-**Lu**-d(GG-TT-CC-AT-GC-AT-GG-AA-CC)	7-Ph-Ph	60.4

[a]The transitions were measured at 260 nm in $NaHPO_4/NaH_2PO_4$ buffer pH 7; 0.03 M [Na^+].

[b]Not determinable.

The phosphoramidites **30-33** were synthesized by the reaction of **22-25** with (2-cyanoethoxy)bis(diisopropylamino)phosphine) in the presence of tetrazole in CH_2Cl_2 with 60-91% yield.

A series of self-complementary oligonucleotides containing 6-phenyllumazine, 7-phenyllumazine, 6-(4-biphenyl)lumazine, and 7-(4-biphenyl)lumazine as modified bases in the different positions were synthesized (Table) using solid-phase phosphoramidite method on Applied Biosystems synthesizer 380B in 0.5 μmol scale applying NPE/NPEOC strategy.[5] The coupling yield of modified phosphoramidites was monitored by colorimetric assay of the released dimethoxy trityl kation, ranging between 95.5% and 99.5%. Purity of the synthesized oligonucleotides was controlled by reversed-phase HPLC and polyacryl gel electrophoresis.

As it follows from the Table, the presence of lumazine bases can influence the stability of self-complementary duplexes in different ways. Incorporation of phenyllumazines at the 5'-end of the strand did not change the stability of duplex, whereas presence of one or two 7-phenyllumazine moieties (sequence 4 and 5) in the middle of the strand increased the stability of duplex for 1.1°C and 1.9°C, respectively. 7-(4-Biphenyl)lumazine, similarly positioned (sequences 10 and 11), raised the melting point for 2.9°C and 9.8°C, respectively. Two 6-phenyllumazine nucleotides (sequence 2) increased the T_m of duplex for 5.1°C, in contrary to 6-(4-biphenyl)lumazine which, probably due to the orientation of bulky biphenyl group, caused the decrease of T_m by 3.1°C (sequence 7) in the first case or hindered the formation of duplex at all in the second case (sequence 8).

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