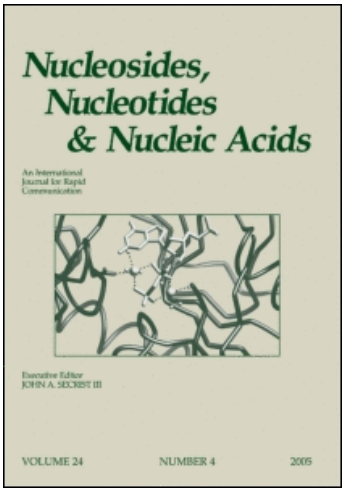

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PHOSPHORAMIDITES AND OLIGONUCLEOTIDES CONTAINING 7-DEAZAPURINES AND PYRIMIDINES CARRYING AMINOPROPARGYL SIDE CHAINS

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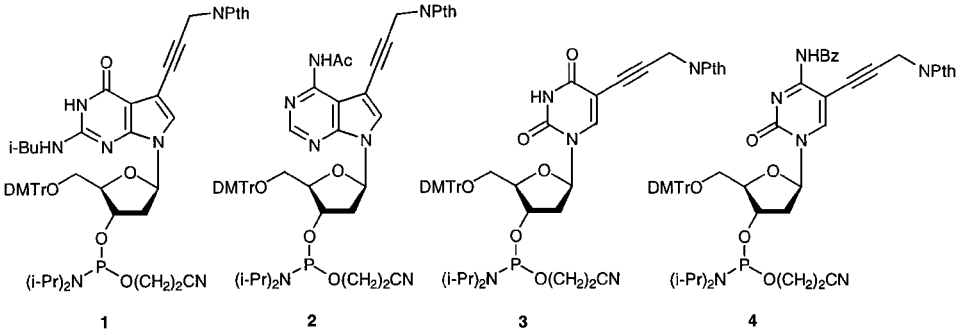
ABSTRACT

The synthesis of phosphoramidites containing 7-deazaguanine, 7-deazaadenine, uracil and cytosine carrying aminopropargyl chains is described. The corresponding oligonucleotides are stabilized in duplexes thermally as well as against degradation by exonucleases.

INTRODUCTION

In a series of manuscripts our laboratory has reported on oligonucleotides containing alkynyl- or aminoalkynyl side chains linked to the 7-position of 7-deazapurines or 8-aza-7-deazapurines (purine numbering is used throughout the manuscript) (1–3). A positively charged side chain transforms a negatively charged oligonucleotide in a zwitterion or even a positively charged species. As a result favorable properties are generated, such as duplex stabilization, resistance against enzymatic degradation, or an increased sensitivity of oligonucleotide detection by MALDI-TOF spectrometry.

*Corresponding author.



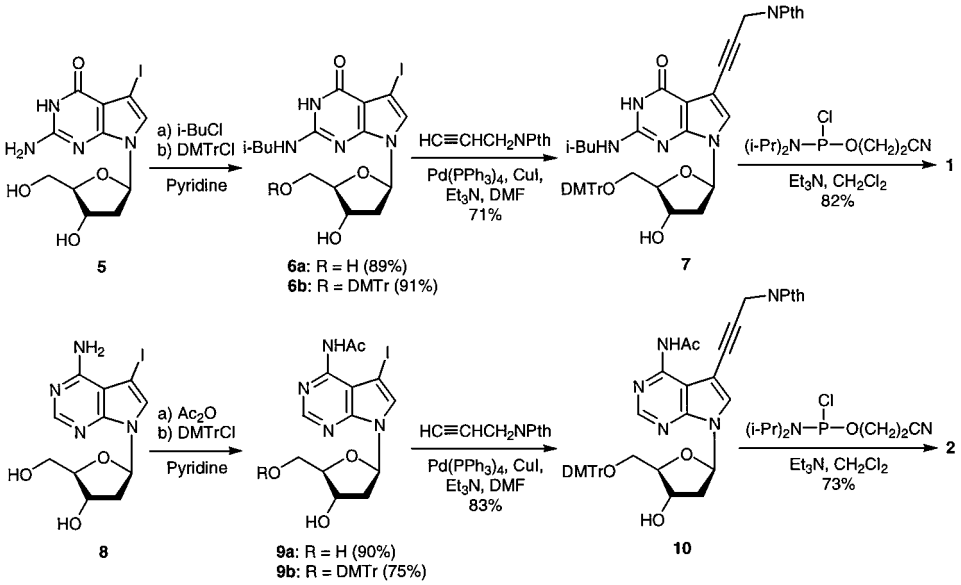
Scheme 1.

This manuscript reports on the synthesis of the phosphoramidites **1–4** (Scheme 1) carrying an aminopropargyl side chain protected by phthaloyl residues (4–6). This protecting group is removed with ammonia under standard conditions (25% ammonia, 60°C, 24 h) and is superior over the rather labile trifluoroacetyl group (7,8) which gives rise to side reaction (9).

RESULTS AND DISCUSSION

*1. Synthesis of the 7-deazapurine Phosphoramidites **1** and **2***

The synthesis of the phosphoramidites **1** and **2** was performed using the iodo compounds **5** (10) and **8** (11) as precursors (Scheme 2). While the 7-deazaguanine



Scheme 2.

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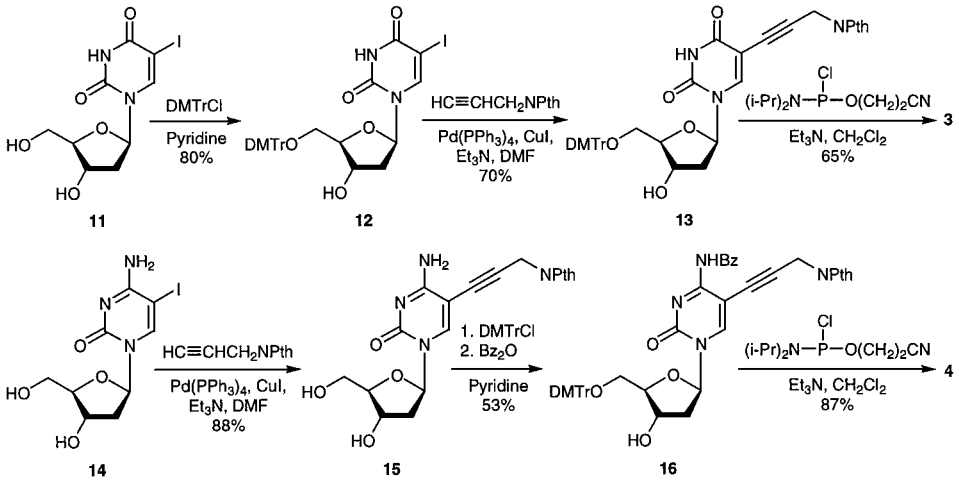

Scheme 3.

base was protected with an isobutyryl residue, the 7-deazaadenine moiety was acetylated. Next, the DMTr residues were introduced. Compounds **6b** and **9b** were coupled with phthaloyl-propargylamine employing the Sonogashira reaction ($Pd(PPh_3)_4$, CuI, Et_3N in DMF). The fully protected nucleosides **7** and **10** gave the phosphoramidites **1** and **2** applying standard methodology.

Table. T_m-Values and Thermodynamic Data of Base Modified Oligonucleotides

Duplex		T_m [°C]	ΔH° [kcal/mol]	ΔS° [cal/mol · K]	ΔG°_{298} [kcal/mol]
5′-d(T-A-G-G-T-C-A-A-T-A-C-T)	**17**	50[a])	−90	−252	−11.8
3′-d(A-T-C-C-A-G-T-T-A-T-G-A)	**18**	47[b])	−89	−253	−10.9
5′-d(T-A-G-G-T-C-A-A-T-A-C-T)	**17**	54[a])	−80	−220	−12.0
3′-d(A-T-C-C-A-**G***-T-T-A-T-**G***-A)	**19**	52[b])	−95	−268	−12.0
5′-d(T-A-**G***-**G***-T-C-A-A-T-A-C-T)	**20**	54[a])	−71	−189	−11.9
3′-d(A-T-C-C-A-**G***-T-T-A-T-**G***-A)	**19**	54[b])	−98	−272	−13.0
5′-d(T-A-G-G-T-C-A-A-T-A-C-T)	**17**	55[a])	−84	−232	−12.4
3′-d(A-T-C-C-**A***-G-T-T-**A***-T-G-A)	**21**	53[b])	−85	−237	−11.8
5′-d(T-**A***-G-G-T-C-**A***-**A***-T-**A***-C-T)	**22**	59[a])	−82	−222	−12.9
3′-d(A-T-C-C-**A***-G-T-T-A*-T-G-A)	**21**	56[b])	−84	−230	−12.7
5′-d(T-A-G-G-**U***-C-A-A-**U***-A-C-T)	**23**	53[a])	−86	−239	−12.0
3′-d(A-T-C-C-A-G-T-T-A-T-G-A)	**18**	50[b])	−91	−254	−11.8
5′-d(T-A-G-G-T-**C***-A-A-T-A-**C***-T)	**24**	57[a])	−70	−187	−12.3
3′-d(A-T-**C***-**C***-A-G-T-T-A-T-G-A)	**25**	56[b])	−87	−239	−13.1
5′-d(T-A-G-G-T-**C***-A-A-T-A-**C***-T)	**24**	54[a])	−81	−222	−12.0
3′-d(A-T-C-C-A-G-T-T-A-T-G-A)	**18**	53[b])	−84	−234	−11.8

[a]) 1 M NaCl, 100 mM $MgCl_2$, 60 mM Na-cacodylate (pH 7.0). [b]) 100 mM NaCl, 10 mM $MgCl_2$, and 10 mM Na-cacodylate (pH 7.0) with 5 μM single strand concentration. The residues with a* represent the modified constituents.

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2. *Synthesis of the Pyrimidine Phosphoramidites **3** and **4***

The reaction sequence performed on the 7-deazapurine nucleosides was also used for the preparation of the phosphoramidite **3** (Scheme 3). The phosphoramidite **4** was prepared in a slightly different way. The 5-iodo-2′-deoxycytidine (**14**) was directly used for the cross coupling reaction yielding the derivative **15.** The crude reaction product was tritylated and benzoylated in a one-pot reaction to give the intermediate **16**. Afterwards, the phosphoramidite **4** was prepared as described before.

3. *Synthesis and Properties of Oligonucleotides*

The oligonucleotides shown in the Table were prepared by solid-phase synthesis in a 1-μmole scale using the phosphoramidites **1**–**4**. The coupling yields were always higher than 96%. The oligonucleotides were deprotected and purified as DMTr derivatives. They were detritylated with 3% aq. trifluoroacetic acid on OPC cartridges. In the case of the dU*-derivatives a side product was formed during the solid-phase synthesis when multiple residues were incorporated. The T_m-values of the Table show that the positively charged aminopropargyl chain stabilizes the duplexes. Also degradation with exonucleases is retarded. The favorable properties of such oligonucleotides were used in MALDI-TOF spectrometry to increase the oligonucleotide detection sensitivity (12).

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