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

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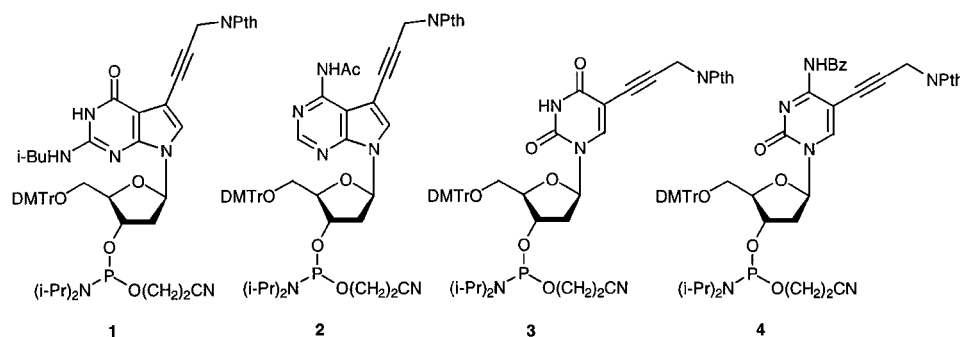
ABSTRACT

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.

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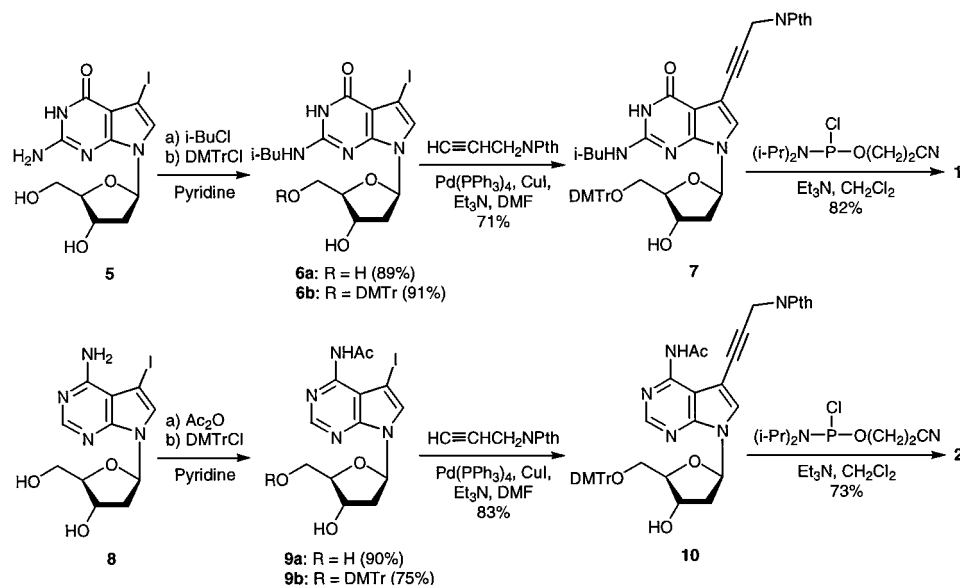
Scheme 1.

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

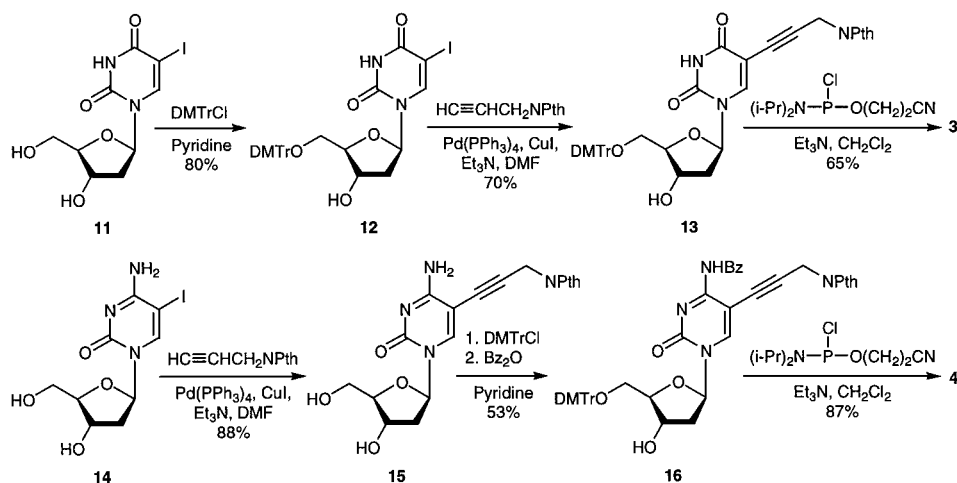
1. Synthesis of the 7-deazapurine Phosphoramidites **1** and **2**

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.





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 ($\text{Pd}(\text{PPh}_3)_4$, 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.



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- μ mole 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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