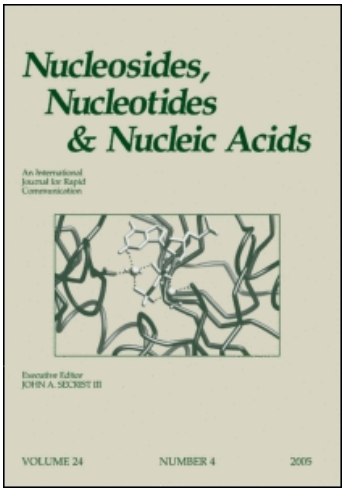

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Positioning of Functionalities in a Heteroduplex Major Groove: Synthesis of 7-Deaza-2-Amino-2'-deoxyadenosines

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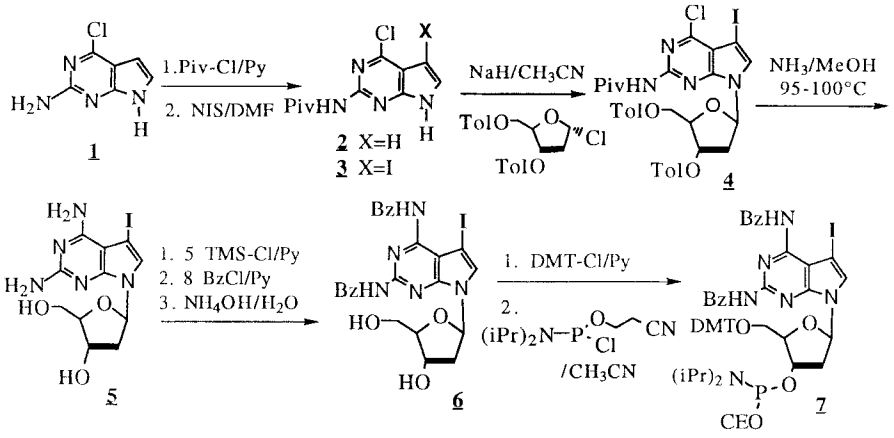
Abstract. *We have synthesized the 7-iodo-, 7-cyano- and 7-propynyl-7-deaza-2-amino-2'-deoxyadenosines and incorporated each into several oligonucleotide (ODN) sequences. These oligonucleotides exhibit enhanced binding affinities to RNA complements relative to unmodified sequences.*

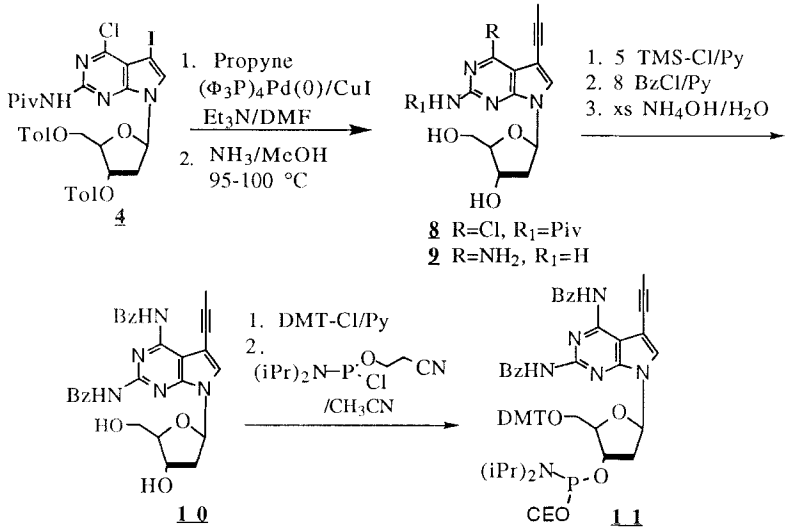
The rationale which explains the forces that contribute to intramolecular stacking of nucleobases in an oligonucleotide bound to a complementary sequence is still controversial. The hydrophobic effect and the interaction of London and dispersion forces have traditionally been invoked in explaining this phenomenon. Recent experiments have postulated that residual negative and positive charges on heterocyclic bases are attractive in nature and are important factors in stacking.[1] Our focus has been to synthesize nucleobases with hydrophobic, polar and polarizable functionalities that should enhance stacking properties with adjacent bases in antisense oligonucleotides.

The intermediate **4** was obtained by the reaction of the 2-amino-4-chloro-7-deaza purine (**1**) with pivaloyl chloride in pyridine followed by the regiospecific iodination[2] with N-iodosuccinimide to yield compound **3**. Sodium-salt glycosylation[3] of **3** yielded the intermediate **4** (60%) as a single β-nucleoside product. Ammonolysis of **4** at 100 °C gave the crystalline 7-deaza-7-iodo-2-amino-2'-deoxyadenosine (**5**) in 65% yield. Transient silylation[4] of the deoxyribose of **5** followed by reaction of the amino groups with benzoyl chloride (8 eq) gave the 2,4-benzoylamino-nucleoside **6**. Routine functionalization of the 5'-hydroxyl group of **6** with dimethoxytrityl chloride, followed by phosphitylation of the 3'-hydroxyl group yielded the phosphoramidite monomer **7**.

The synthesis of the 7-deaza-7-propynyl-2'- deoxyadenosine **9** likewise relied on the intermediate **4**. A regiospecific palladium-catalyzed coupling of propyne to the 7-deaza-7-iodo carbon was effected using *tetrakis* - triphenyphosphine palladium(0)-copper(I) iodide catalyst combination.[5] Using a 10 mol % of Pd(0) catalyst and a ratio of Pd(0)/Cu(I) of 1:2 gave optimum yields of **8** (85%) from an overnight reaction at room temperature. The material **8** was further reacted with ammonia at 100 °C to yield the 2,4-diamino compound **9** in 55% yield. Transient silylation[5]



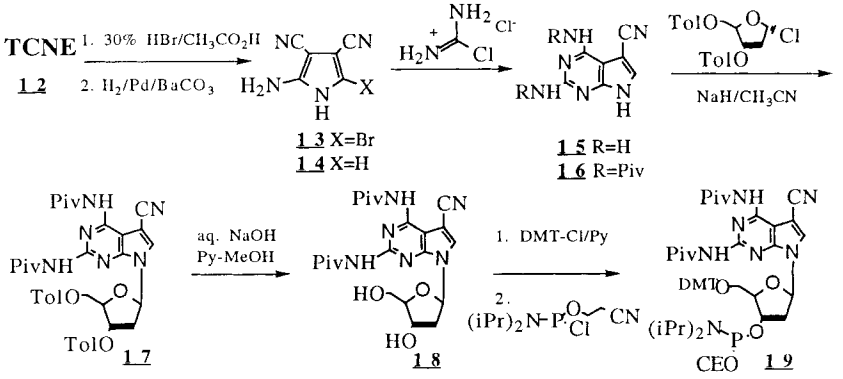
Scheme 1



Scheme 2

of 9 followed by a reaction with 5 equivalents of benzoyl chloride gave the compound 10. Functionalization of the 5'-hydroxyl with dimethoxytrityl chloride and subsequent phosphitylation yielded the phosphoramidite monomer 11.

Annulation of tetracyanoethylene in 30% HBr in acetic acid yielded the dicyanopyrrole 13. This material was reduced using Pd on $BaCO_3$ and then annulated using chloroformamidine hydrochloride[6] at 170° C to yield the 2-amino-7-deazaadenine 15. Acylation of 15 using pivaloyl chloride



Scheme 3

Table 1. Effect on melting temperature of ODN sequences substituted with 7-deaza-2-amino-2'-deoxyadenosines.

CTCGTACCATTCCGGTCC	GGACCGGAAGGTACGAG	ACCGAGGATCATGTCGTACGC
7-Iodo (7) +3.05* (0.84)#	+1.30* (-0.03)#	+0.57* (-0.15)#
7-Cyano (19) +2.78* (0.54)#	+0.37* (-0.98)#	+0.28* (0.16)#
7-Propynyl (11) +3.86* (1.65)#	+1.54* (0.19)#	+0.85* (0.12)#

* Effect of incorporation of modified heterocycles *vs* 2'-deoxyadenosine
\# Effect of I , CN or propynyl functionalities *vs* 2,6-diamino-2'-deoxyadenosine

(5eq) in pyridine yielded a product bearing a single pivaloyl group on each exocyclic amine, **16**. A sodium-salt glycosylation yielded the 2,4-di-pivaloylamino β–nucleoside **17** was followed by a selective deprotection of the toluoyl groups of **17** using a 4N aqueous solution of sodium hydroxide in methanol-pyridine (5:30:65) at RT to yield **18**. Nucleoside **18** was subsequently derivatized with dimethoxytrityl chloride and then with 2-cyanoethyl-N,N-diisopropyl chlorophosphoramidite to yield the phosphoramidite **19.**

Incorporation of monomers **7**, **11** or **19** in sequences for spectroscopic melting studies (Tm) was accomplished with the finding that each coupled with d(C, G, A) and T derivatized resins with greater than 90% efficiency, as measured by trityl cation yields. Oligonucleotide sequences containing one incorporation exhibited a 3-4° improvement in melting temperatures over identical sequences containing 2'-deoxyadenosine (Table 1). Five incorporations in a 17-mer or 21-mer improved binding by 0.28-1.54°/modification and thus, the effect of

multiple incorporations was not additive for **7**, **11** or **19**. The isolated effect of the iodo, propynyl or cyano groups was small and positive for a single incorporation; small and negative for multiple incorporations, as compared to 2-amino-2'-deoxyadenosine. A notable exception was the effect of the propynyl group, which was positive for all sequences melted. At this time we can offer no explanation for why multiple incorporations do not additively enhance Tm.

Molecular modeling of the 7-propynyl nucleoside suggests that this group is well tolerated in the context of an A-form duplex and the propyne group is positioned well over pyrimidine bases on the 3'-end. Additionally, molecular mechanics calculations, using a 7-propynyl-7-deaza-2-amino-2'-deoxyadenosine hydrogen bound to a uridine suggests that favorable stacking interactions and not Watson-Crick hydrogen bonds are responsible for the enhanced binding affinity which is observed. The results of these calculations and an account of the biological activity of antisense oligonucleotides using these nucleobases will be published elsewhere.

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