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A Novel 2'-Deoxynucleoside Designed for Enhanced Recognition of A.T. Base-pairs.

Stanislas Czernecki*, Antoine Hoang and Jean-Marc Valéry

Laboratoire de Chimie des Glucides, Université Pierre et Marie Curie,
4 place Jussieu, 75005 Paris, France

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Abstract : The title base modified purine deoxynucleoside **1** , designed to be incorporated in triplex-forming oligodeoxynucleotides selectively binding to the A.T. base pair, was prepared by non ionic azido-phenylselenylation reaction applied to a 6-vinylpurine substrate.

The existence of triple helical nucleic acids is an old observation[1] and has recently been documented for synthetic oligodeoxynucleotides[2] which are capable of binding to regions of double helical DNA through the formation of localized triplex structures.[3] This recognition process has attracted considerable interest because of its potential use in regulating gene expression.[4] However, to date, some limitations are encountered, and the choice of the target sites in DNA are limited to homopurine sequences. In order to supress this constraint and to allow selective binding to any chosen sequence, several base-modified nucleosides were previously introduced in triple-helix-forming oligonucleotides both to improve binding or to extend the range of recognition sequences.[4]

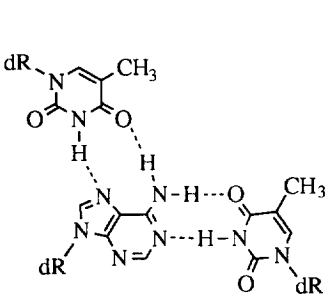
As part of a program devoted to propose solutions to this problem, we undertook the synthesis of base-modified nucleosides able to establish hydrogen bonds with the two bases of a base pair of DNA instead of one, like in classical recognition process. This could be achieved by introducing a chain containing hetero atoms suitably chosen and placed. This concept is illustrated in Figure 1 for the A.T. base pair.

We decided to take advantage of the second hydrogen bond acceptor site present in the thymine base (Fig. 1b) and the novel 2'-deoxy nucleoside, 9-(2-deoxy-β-D-*erythro*furanosyl)-6-(2-*N*-acetylaminoethyl)-2-amino-purine **1**, was thus designed[5] to contain a complementary donor-acceptor-donor array and to form the base triplet shown in Figure 1c.[6]

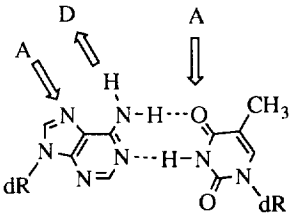
We report herein the synthesis of compound **1**. Moreover the derivative **2**, protected at the 2-amino group of the purine base, was prepared in order to allow efficient oligonucleotide synthesis.

As outlined in the Scheme,[7] the key step of our strategy was the introduction of the primary amine - masked as an azido group - at the *terminal* position of the 2',3'-di-*O*-acetyl-6-vinyl-2'-deoxyguanosine **6**. This compound was obtained in three steps from commercially available 2'-deoxyguanosine **3**. The vinyl residue was grafted at C-6 by means of a cross coupling reaction between the 6-*O*-tosyl derivative **5** and tetravinyltin catalyzed by the $Pd(PPh_3)_4$ - LiCl system.[8]

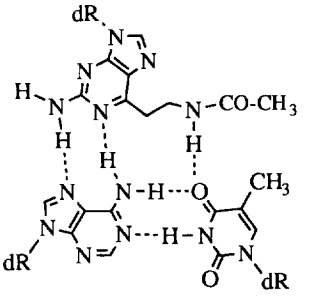
Figure 1



(a) TxA.T. base triplet
dR = 2-deoxyribose

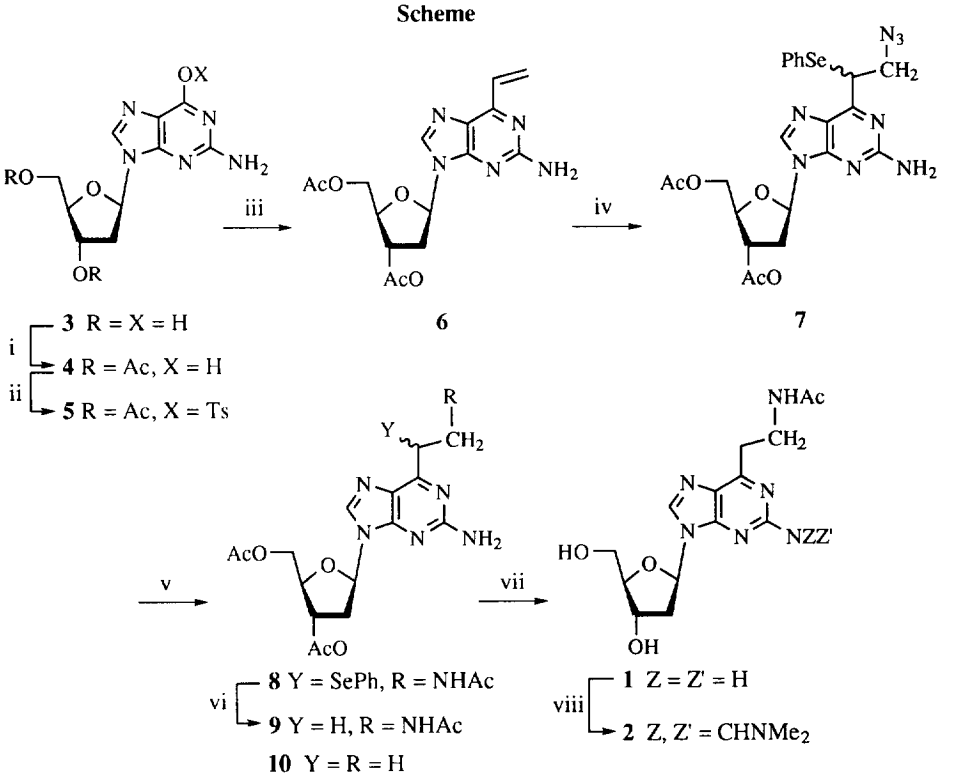


(b) major groove hydrogen bonding sites of A.T. base pair



(c) 1xA.T. base triplet

Scheme



Reagents and conditions: i; Ac_2O, DMAP, Et_3N, MeCN. ii; K_2CO_3, TsCl, MeCN, reflux. iii; see ref.8. iv; Me_3SiN_3, *N*-PSP, CH_2Cl_2, 45 min.. v; PPh_3, Ac_2O, toluene, 60 °C, 2 h. vi; H_2, Ni Raney, THF, 45 min. vii; NaOMe, MeOH, 20 min. then IRN 77 (H^+ form). viii; $Me_2NCH(OMe)_2$ 15 equiv., DMF, 70 °C.

Introduction of the azido moiety at the less substituted position of the vinyl group was obtained by means of a non-ionic azidophenylselenylation.[9] The use of the Me_3SiN_3 / *N*-phenylselenophtalimide (*N*-PSP) combination allowed us to prepare compound **7** in 71 % yield. Next, the P-N ylide obtained by Staudinger reaction was treated *in situ* by acetic anhydride to afford the *N*-acetamide **8** (89 % yield), which was submitted to hydrogenation over Raney Nickel.[10] Hydrogenolysis of the C-6'-SePh bond yielded **9** (90 %).[11] The same hydrogenation procedure, in the presence of acetic anhydride, was applied to compound **7** in order to effect both the reduction of the C-Se bond and the transformation of the azido group into the *N*-acetylamino moiety in a single step. If the formation of compound **9** was effectively observed, the transformation afforded, in equal amounts, another product which was identified as the derived 2-amino-6-ethylpurine deoxynucleoside **10**. The formation of this compound could be explained by elimination of either "$PhSeN_3$" or "HN_3" followed by *in situ* hydrogenation and (or) hydrogenolysis of the resulting product . The first possibility was already reported in the literature for selenoglycosides[12] and the second one quoted in an alicyclic series.[9b]

The end of the sequence was straightforward: the deprotection of the hydroxyl groups was conducted as usual to give compound **1**. The reaction with phenoxyacetyl chloride, although successfully used for protection of the 2-amino function in either adenosine or guanosine,[13] proved to be ineffective when applied to **1**.

On the contrary, the *N*-dimethylaminomethylene group, which was already claimed to be suitable for preparation of diribonucleoside phosphates,[14] was easily introduced by heating the free nucleoside together with dimethylformamide dimethylacetal in DMF solution, thus affording in quantitative yield the protected derivative **2**, ready for further oligonucleotide synthesis.

In conclusion, this work has demonstrated the usefulness of azidophenylselenylation as an efficient tool for selective transformation of the base in nucleoside chemistry. Further applications of this methodology are in progress.

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11. Following physical data are representative: Compound **9**: mp = 145-147 °C. $[\alpha]_D^{20} = +2$ (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$) δ = 1.90 (s, 3H); 2.00 and 2.10 (2s, 2x3H); 2.47 (ddd, 1H); 2.95 (ddd, 1H); 3.15 (m, 2H, J= 6.2 Hz); 3.65 (m, 2H, J= 6.2 Hz); 4.30 (m, 3H); 4.95(s, 2H); 5.40 (dt, 1H, J= 6.2 and 2.3 Hz); 6.30 (dt, 1H, J= 7.9 and 2.3 Hz); 6.70 (s, 1H); 7.80 (s, 1H). Compound **1**: $[\alpha]_D^{20} = -6.5$ (*c* 0.15, MeOH). ^1H NMR (250 MHz, D_2O) δ = 1.80 (s, 3H); 2.50 (ddd, 1H); 2.85 (ddd, 1H); 3.10 (t, 2H, J= 6.1 Hz); 3.60 (t, 2H, J= 6.1 Hz); 3.80 (m, 2H, J= 13.3 and 3.6 Hz); 4.25 (q, 1H, J= 3.6 Hz); 4.65 (m, 1H, J= 6.8 and 3.6 Hz);6.40 (t, 1H, J= 6.9 Hz); 8.20 (s, 1H).
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