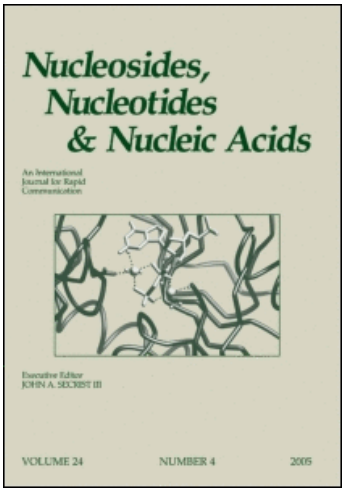


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# Synthesis of Amide Linked Nucleosides at the 6 Position of Deoxy Inosine and Their application to DNA synthesis, Hybridization Studies.

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# Synthesis of amide linked nucleosides at the 6 position of deoxy inosine and their application to DNA synthesis, hybridization studies.

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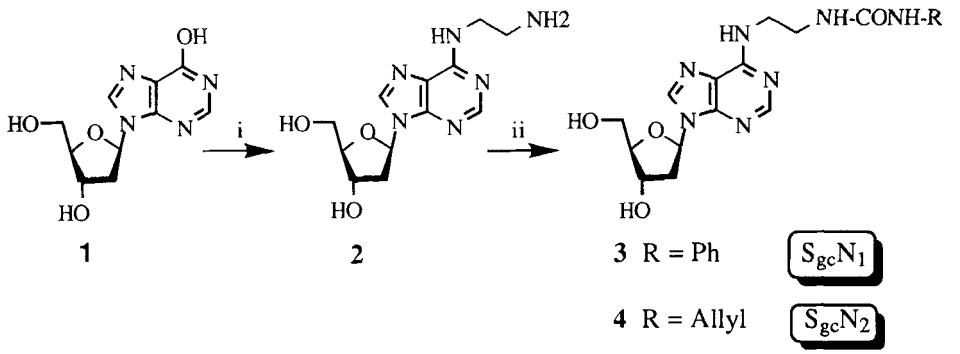
**Abstract:** Synthesis of amide linked nucleosides at the 6 position of the purine base to recognize "G:C" base pair in a DNA duplex is described. Here we describe the synthesis of amide linked nucleosides containing heteroatoms N, O and C and their application to DNA synthesis and hybridization studies.

The existence of triple helical structures in nucleic acids is an old observation and has been documented for synthetic oligodeoxynucleotides (ODNs) which are capable of binding regions of double-stranded DNA through the formation of localized triplex structures.

Triple helix formation relies upon formation of hydrogen bonds between bases in the third strand and purines already engaged in Watson-Crick hydrogen bonding with pyrimidines in the major groove of the DNA duplex. Although this recognition process has attracted considerable interest, some important limitations are encountered.

As a part of our programme devoted to propose solutions to this problem we decided to design and synthesize modified "Super" nucleosides able to establish hydrogen bondings with the two bases of a base pair of DNA instead of one and we report herein our results for the "G:C" base pair recognition.We have planned to take advantage of the remaining hydrogen bonding sites of the guanine base.

**Synthesis of N-linked nucleoside:**

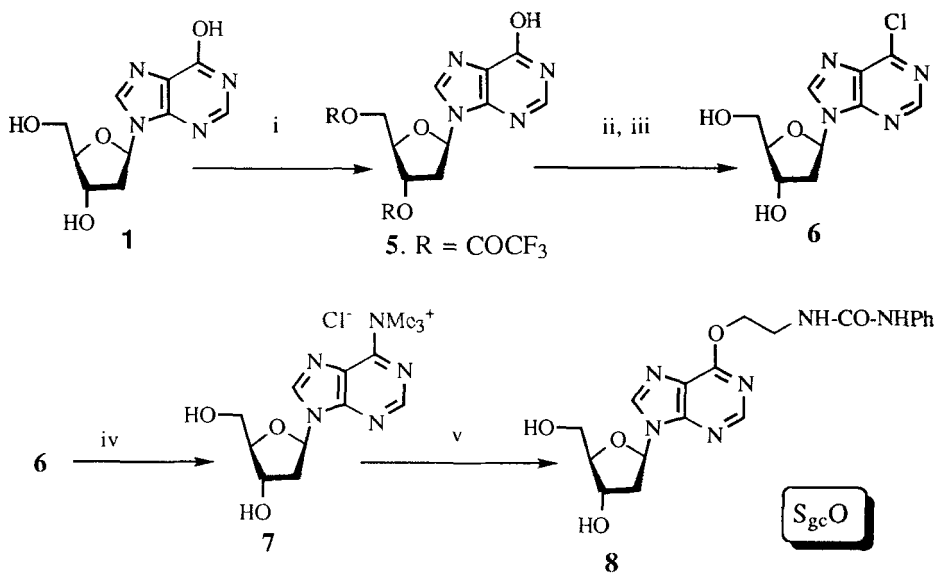



Reagents and conditions: (i) HMDS, 1,2-diaminoethane, PTSA cat., 50% (ii) R-N=C=O, MeOH 70%.

**Scheme 1**



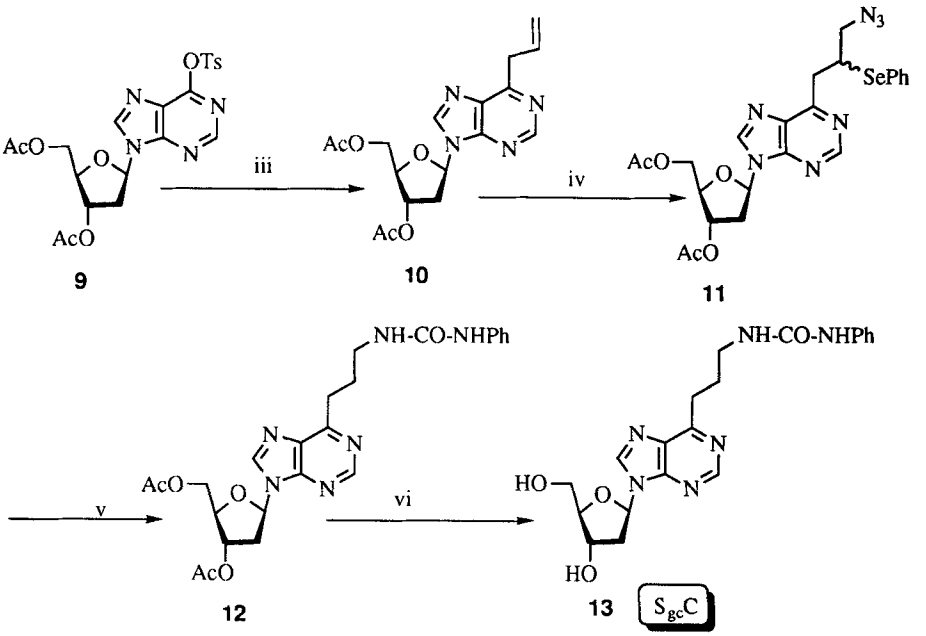
**Synthesis of O- linked nucleoside:**




Reagents and conditions: (i) $(CF_3CO)_2$, $CH_2Cl_2$. (ii) $SOCl_2$, DMF, $CH_2Cl_2$. (iii) MeOH, Alumina ( 3 steps 75%) (iv) 1,2-dimethoxyethane, $Me_3N$, $NEt_3$ (v) HO-$CH_2$-$CH_2$-NH-CO-NH-Ph, DBU, DMF ( 2 steps 50% ).

**Scheme 2**

**Synthesis of C-linked nucleoside:**




Reagents and conditions: (i) $Ac_2O$, $NEt_3$, DMAP, acetonitrile, 95% (ii) TsCl, $K_2CO_3$, acetonitrile, 70% (iii) ( $CH_2$=CH-$CH_2$)$_4$Sn, Pd($PPh_3$)$_4$, LiCl, dioxane, 55% (iv) $(PhSe)_2$, $NaN_3$, PhI(OAc)$_2$, $CH_2Cl_2$, 50% (v) $nBu_3SnH$, $BEt_3$, Benzene then Ph-N=C=O, 60% (vi) NaoMe, MeOH, 72%.

**Scheme 3**

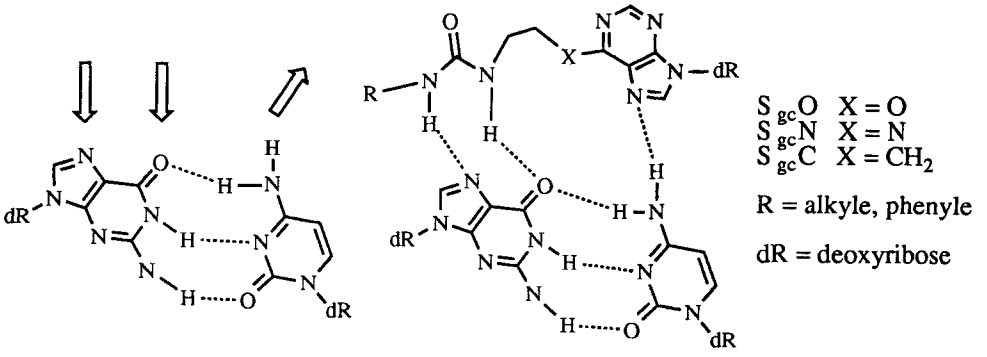



**FIGURE 1**

3'- TGTCAA TTCTT CTT X TTCTTAACTCG -5'
5'- ACAGTT AAGAAGAAY AAGAATTGAGC -3'
5'- TTCTT CTT Z TTCTT- 3'

FIGURE 2

**TABLE 1:** $T_m$ - Values ( ° C ) of Triplex formation of Oligonucleotides

| Olig-nucleotide | X•Y Z | T•A | C•G | A•T | G•C |
|---|---|---|---|---|---|
| unmodified | a) A | 12 | 23 | 13 | 11 |
| | b) C | <5 | 39 | 11 | 17 |
| | c) G | <5 | 15 | 23 | 13 |
| | d) T | 31 | 18 | <5 | 20 |
| modified | e) 8 | 22 | 31 | 27 | 22 |
| | f) 3 | 15 | 18 | 20 | 21 |
| | g) 4 | 14 | 14 | 17 | 19 |
| | h) 13 | 11 | 13 | 18 | 17 |



Thus the series of modified 2'-deoxy nucleosides $S_{gc}$ were designed. Synthesis of nucleoside analogues for DNA synthesis were described in Scheme 1, 2 and 3. The derived ODNs are expected to be involved in a triplex structure where the purine will face the cytosine while the urea residue will bind to the acceptor sites of the guanine ( Fig 1). Such structure could be quated as G:C*S. These nucleosides have been incorporated in the 14 mer ODNs **"e - h"** using standard phosphoramidite methodology.

A Preliminary assay of triplex stability was made using a 26 mer double stranded target sequence ( FIG 2).

The Tm values were measured in a buffer at pH 6.0 (Sodium cacodylate 20 mM, Magnesium Chloride 10 mm and Sodium Chloride 100 mM).

From the results listed above, one may notice that the stability of the triplex decreased in the order $S_{gc}O > S_{gc}N > S_{gc}C$ (Table 1). Furthermore, the results obtained with the N-linked derivatives showed that, compared to the rather flexible allyl chain, the phenyl group provided a noticeable increase of the Tm value (2-3°C).

## REFERENCES AND NOTES

1. For leading references, see: Thuong, N. T.; Hélène, C. *Angew. Chem. Int. Ed. Engl.*, **1993**, *32*, 6666-6690.

2. For a similar strategy, see Zimmerman, S.C.; Schimdt, P. *J. Am. Chem. Soc.*; **1995,** *117*, 10769-10770.

3. Dr. J. S. Sun (Laboratoire de Biophysique, Museum National d'Histoire Naturelle, Paris, France) is gratefully acknowledged for achieving the molecular modelling studies which resulted in the design of Sgc and Tm measurments.

4. Dr. N. T. Thuong and Dr. U. Asseline (CBM-CNRS) Université d'Orléans, France, are gratefully acknowledged for performing the synthesis of the ODN.