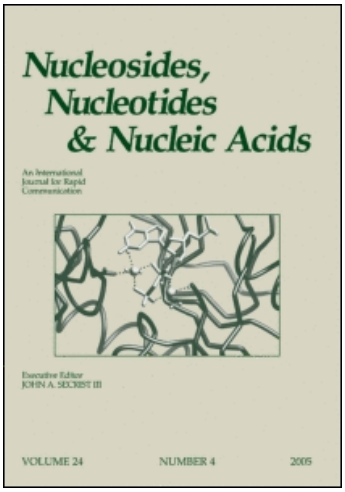

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IMIDAZOLE-4-CARBOXAMIDE AND 1,2,4-TRIAZOLE-3-CARBOXAMIDE DEOXYNUCLEOTIDES AS SIMPLIFIED DNA BUILDING BLOCKS WITH AMBIGUOUS PAIRING CAPACITY

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Abstract: 2'-Deoxynucleosides of imidazole-4 (or 1,2,4-triazole-3)-carboxamide, ethyl imidazole-4 (or 1,2,4-triazole-3)-carboxylate were synthesized by enzymatic glycosylation using N-deoxyribosyltransferase from a lactobacterium. The base pairing properties of Y and V when placed opposite the natural DNA bases as well as their self were evaluated by thermal denaturation experiments. DNA templates containing imidazole-4-carboxamide base were used in elongation reaction catalysed by Klenow fragment.

3-Nitropyrrole and 5-nitroindole designed to serve as universal base were recently reported.[1] Hydrophobic nucleosides isosteres of pyrimidines and purines to base pair in DNA were also investigated.[2] Although these nucleosides have had some interesting applications (especially as primers for dideoxy sequencing and PCR), the need to find base analogues that allow non-discriminate hydrogen-bonding to each of the four natural bases and non-too-destabilizing in a duplex are still in progress. Another field of investigations consists in the design of purine and pyrimidine base analogues that have ambivalent hydrogen-bonding modes.[3] Such bases, used in the DNA template or as triphosphate, may find applications in mutagenesis.[4]

In an earlier study,[5] we proposed as mutagenic nucleoside a simplified purine analogue, 5-amino-imidazole-4-carboxamide (Z), expected to pair with canonical bases owing to the rotations of its carboxamide group and around its glycosidic bond. In the following report,[3d] we described the synthesis of 1-(2-deoxy-β-D-ribofuranosyl)imidazole-4-carboxamide (noted dY). Such imidazole-4-carboxamide nucleotides (dZTP, dYTP and alkylamide analogues $dY^{Me}TP$, $dY^{Pr}TP$) were found to be substrate for DNA polymerases (Klenow fragment of DNA polymerase I, Sequenase, *Taq* DNA polymerase). A detailled study showed that the resulting incorporation depends on the polymerase used for the elongation reaction and the base in the DNA template opposed

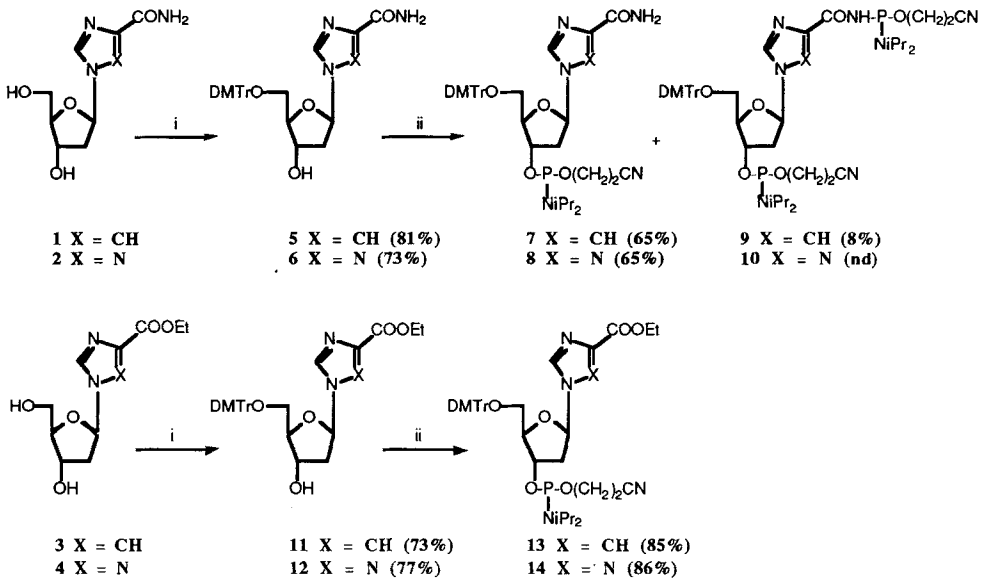
to the incoming triphosphate.[6] Thus, dYTP was incorporated by the Klenow fragment deficient in 3'-exonuclease activity (KFexo-) opposite T and G in DNA templates, while $dY^{Me}TP$ and $dY^{Pr}TP$ were incorporated opposite A and G. Elongation using HIV-1 RT resulted in the incorporation of dYTP face to A, and of $dY^{Me}TP$ and $dY^{Pr}TP$ face to A and T. Incorporation catalyzed by the KFexo- and HIV-1 RT was efficient in both cases, but lower compared with the incorporation of standard bases into correct sites.

In this report, we describe the synthesis of two heterocyclic carboxamide nucleosides, imidazole-4-carboxamide (Y) and 1,2,4-triazole-3-carboxamide (V). The thermal stability of heteroduplexes containing Y and V residues was measured. The analysis of incorporated nucleotides opposite Y by KFexo- is also reported.

Synthesis of nucleosides and oligonucleotides

1-(2-deoxy-β-D-ribofuranosyl)imidazole-4-carboxamide (**1**) and ethyl 1-(2-deoxy-β-D-ribofuranosyl)imidazole-4-carboxylate (**3**) were prepared *via* enzymatic transglycosylation as described previously.[3d] 1-(2-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (**2**) was obtained by 2'-deoxygenation of ribavirin (in 4 steps) or by treatment with ammonia/methanol of ethyl 1-(2-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate (**4**) which was obtained by enzymatic transglycosylation from ethyl 1,2,4-triazole-3-carboxylate.

The nucleosides (**1**-**4**) were 5'-dimethoxytritylated and phosphitylated following standard procedures (Scheme). In the case of carboxamide derivatives (**1** and **2**), phosphitylation with 2-cyanoethyl-*N,N,N',N'*-tetraisopropylphosphoramidite resulted in the formation of 3'-phosphitylated compounds (**7** and **8**) and a small amount of diphosphitylated products (**9** and **10**).[7] Such a side reaction with a carbamoyl group was observed during the phosphitylation of peptides containing serine or tyrosine amides. The analogue phosphoramidites were used to prepare modified oligomers on a Expedite Millipore DNA synthesizer. A 0.15 M to 0.20 M solution was used as compared with the usual 0.1 M solution phosphoramidites and a coupling time for modified bases increased to 5 min on a 1 μmol scale. Using the ester derivatives (**13**,**14**) good coupling yields were obtained (~95%), whereas with the carboxamide phosphoramidites (**7**,**8**) a lower efficiency was obtained (coupling yields for Y and V were 72-77%). The crude sequences were purified by reverse phase HPLC at pre- and post-DMTr removal stages. Short sequences containing up to three Y residues could be obtained in acceptable yields using **7**. A long sequence (50-mer) containing five Y residues was successfully synthesized using **13**. Incorporation of Y and V bases into oligomers was confirmed by HPLC analysis of nucleosides from enzymatically digested oligomers and mass electrospray.



Reagents and conditions: (i) DMTrCl/pyridine (ii) 2-cyanoethyl-*N,N,N',N'*-tetraisopropylphosphoramidite (2 eq.) and diisopropylammmonium tetrazolide (0.5 eq.) in CH_3CN.

Scheme

Melting profiles

Imidazole-4-carboxamide (Y) and 1,2,3-triazole-3-carboxamide (V) were incorporated into heptadecamer sequences at the 9th position since substitution in the centre had a more detrimental effect for the modified bases previously studied such as 3-nitropyrrole and 4-, 5-, or 6-nitroindole.[3a] The melting temperatures (Tm) values of duplexes composed of 5'-d(CAAAATGGMGGCCAAGT)-3' and 5'-d(ACTTGGCCNCCATTTTG)-3' (M=N= Y,V,A,C,G,T) are listed in Table. In all cases, sharp melting transitions were observed indicating cooperativity in their thermal dissociations-associations (Figure 1). The heteroduplexes containing the modified base Y are less stable than the fully complementary A:T and C:G duplexes. However, the destabilizing effect induced by the introduction of one Y residue depends on the base opposite it. Thus, the G:Y base pair induces a slight destabilization (54°C) as compared to the A:T base pair. When two substitutions of Y were made opposite G, destabilization was 8°C as compared to one substitution. Similar melting curves were obtained with oligomers containing the V base (not shown). All the Tm values are in the range of 47 to 54°C, similar to the values for natural mismatches (48-55°C). This may indicate a significant stabilization of heteroduplex through base stacking interactions.

Table: Tm (°C) of the duplexes :
5'-d(CAAAATGG**M**GGCCAAGT)-3'
3'-d(GTTTTACC**N**CCGGTTCA)-5'
measured 00 in .1 M NaCl, 10 mM sodium cacodylate (pH 7.0) at 1 μM of each strand concentration.

M:N	Tm	M:N	Tm	M:N	Tm
A:T	58	V:A	50	A:A	50
C:G	59	V:G	52.5	G:G	55
A:Y	50	A:V	51	A:G	54
G:Y	54	G:V	53	G:A	52
C:Y	47	C:V	47.5	C:A	49.5
T:Y	51.5	T:V	50.5	G:T	54.5
Y:T	52	V:T	50	T:T	50.5
Y:Y	47.5	V:V	47.5	C:T	48.5
$(G:Y)_2$*	46	T:A	57.5		

* 5'-d(ACTTG**Y**CC**Y**CCATTTTG)-3'

Otherwise we notice a preferable pairing of Y and V with G with a slight destabilization of the heteroduplexes, and can range the base pairing stability as G:N>T:N≈A:N>C:N≈N:N, N being Y or V. This is to compare with the relative order of helix stability observed for hypoxantine (I) base pairs: I:C>I:A>I:T>I:G and pleads in favour of a preferable A-like orientation of Y and V into oligonucleotides in solution. During this work, a similar observation was noted by Bergström et al. using a self-complementary dodecamer, although in the context the order was T:Y>G:Y>A:Y>C:Y.[3f]

Incorporation of dNMP opposite Y catalyzed by the Klenow fragment of DNA pol I

In order to determine the response of the Klenow fragment of DNA poymerase I to nucleotide analogues in the template strand, we examined the product of extension of a 5'-^{32}P-labeled primer hybridized to a template containing one or more Y residues. Using a 14-mer containing three successive Y residues [5'-d(**YYY**GCATGAGCTGC)-3'] as template and a ^{32}P-labeled 11-mer [5'-d(GCAGCTCATGC)-3'] as primer, addition of a single dNMP by KFexo- was observed in all cases (Lanes 8: primer; 9: dATP; 10: dCTP; 11: dGTP; 12: dTTP; 13: dYTP; 14: 4 dNTPs), and the N+2 product was detected in the case of dTTP. Complete elongation was not observed under these conditions. When the template:primer hybrid was elongated with the Klenow fragment, degradation of the

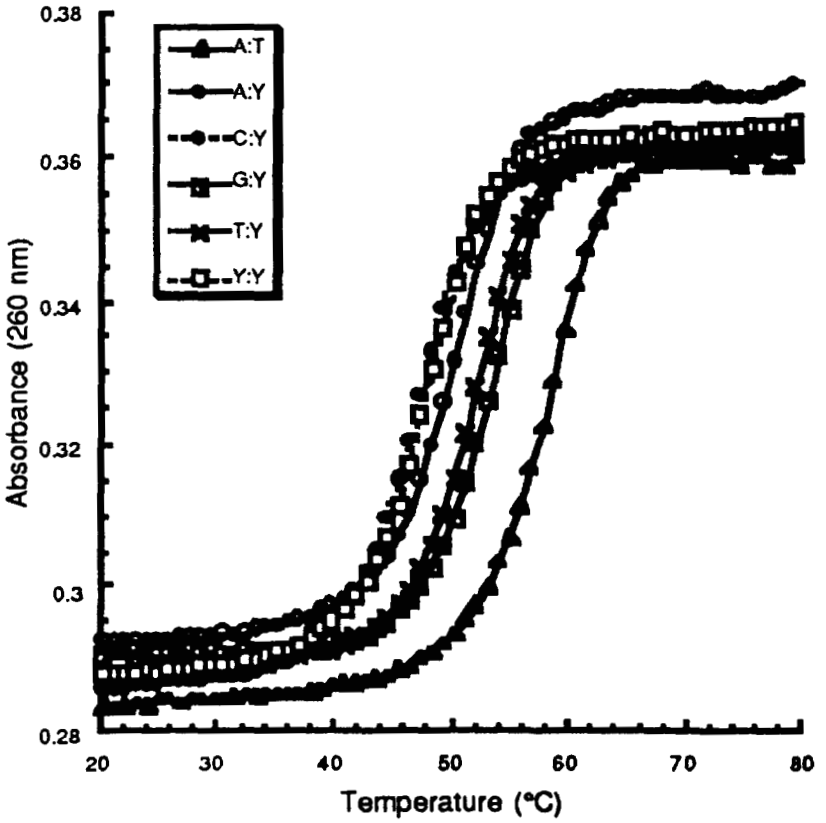


Figure 1 : Thermal melting curves of
5'-d(CAAAATGG**M**GGCCAAGT)-3'
3'-d(GTTTTACC**N**CCGGTTCA)-5'
with **M** being either A,C,G,T or Y and
N being Y or T.

primer was observed. The N+1 product was formed only with dTTP, suggesting that the exonuclease activity differentiated the Y:N base pair formed (Figure 2, (Lanes 1: primer; 2: dATP; 3: dCTP; 4: dGTP; 5: dTTP; 6: dYTP; 7: 4 dNTPs). Using 5'-d(CAAAATGGYGGGCAAGT)-3' as template and 5'-^{32}P-d(ACTTGGCC)-3' as primer, chain extension catalyzed by the KFexo- was efficient opposite Y (Figure 3): the N+1 products were synthesized with dATP (lane 2), dGTP (lane 4), dTTP (lane 5) or dYTP (lane 6), and the N+3 product was formed with dCTP (lane 3). When a mixture of the four dNTPs were added, the full 17-mer was synthesized (lane 7). In the conditions used, the efficiency of elongation opposite Y was T>A>G,Y>C.

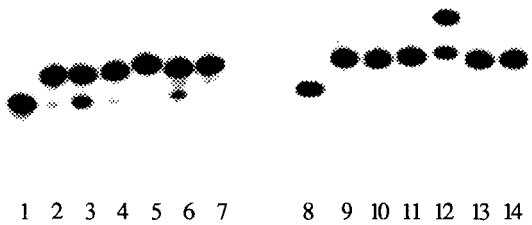


Figure 2

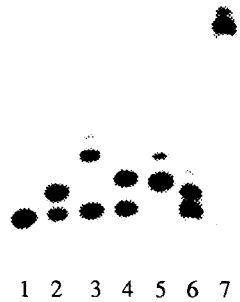


Figure 3

Conditions: Appropriate templates (15-30pmol, 0.75-1.5μM) and [γ-^{32}P]-labeled primers (15pmol, 0.75μM) in 20ml of 2 x pol. buffer (20mM Tris-HCl, pH 7.5, 10mM $MgCl_2$, 15mM DTT) were heated at 75°C for 15 min, then cooled slowly to room temperature over 1 hour. Final reaction mixtures (5μl) contained 0.3-0.4μM annealed primer-template, 0.6U KF- or 1.2U KF, and 50μM dNTP in the corresponding polymerase buffer. Reactions were carried out at 37°C for 20 min, quenched by addition of loading buffer, then analysed by PAGE (20%, 7M urea).

These results indicate that the base Y in the template strand led to non-specific incorporation and does not cause a block to replication after dNMP incorporation, even when incorporated opposite a base Y in the template.

We recently reported *in vitro* mutagenesis using dYTP and dY^{Pr}TP.[4b] These triphosphates were incorporated as deoxypurine analogues, more efficiently as a dATP than as dGTP analogues. Once incorporated into a DNA template, their ambiguous hydrogen bonding potential gave rise to both transitions and transversions (11-15%), at frequencies of 3-4% per base per amplification. These results showed altogether that dY causes mutations by writing an ambiguous base and then reading the template containing it through alternative pairing schemes.

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REFERENCES

1a. Nichols, R.; Andrews, P.C.; Zhang, P.; Bergstrom, D.E. *Nature* **1994** ***369***, 492-493. 1b. Loakes, D.; Brown, D.M. *Nucleic Acids Research* **1994** ***22***, 4039-4043. 1c. Bergstrom, D.E.; Zhang, P.; Toma, P.H.; Andrews, P.C.; Nichols, R. *J. Am. Chem. Soc.* **1995** ***117***, 1201-1209. 1d. Loakes, D.; Brown, D.M.; Linde, S.; Hill, F. *Nucleic Acids Research* **1995** ***23***, 2361-2366. 1e. Van Aerschot, A.; Rozenski, D.; Loakes, D.; Pillet, N.; Schepers, G.; Herdewijn, P. *Nucleic Acids Research* **1995** ***23***, 4363-4370. 1f. Amosova, O.; George, J.; Fresco, J.R. *Nucleic Acids Research* **1997** ***25***, 1930-1934. 1g. Bergstrom, D.E.; Zhang, P.; Johnson, W.T. *Nucleic Acids Research* **1997** ***25***, 1935-1942.

2a. Schweitzer, B.A.; Kool, E.T. *J. Org. Chem.* **1994** ***59***, 7238-7242. 2b. Schweitzer, B.A.; Kool, E.T. *J. Am. Chem. Soc.* **1995** ***117***, 1863-1872.
3a. Brown, D.M.; Kong Thoo Lin, P. *Carbohydrate Res.* **1991** ***216***, 129-139. 3b. Brown, D.M.; Kong Thoo Lin, P. *Nucleic Acids Research* **1992** ***20***, 5149-5152. 3c. Bergstrom, D.E.; Zhang, P.; Zhou, J. *J. Chem. Soc. Perkin Trans I* **1994**, 3029-3034. 3d. Pochet, S.; Dugué, L.; Meier, A.; Marlière, P. *Bioorg. Med. Chem. Lett.* **1995** ***5***, 1167-1684. 3e. Bergstrom, D.E.; Zhang, P.; Johnson, W.T. *Nucleosides and Nucleotides* **1996** ***15***, 59-68. 3f. Johnson, W.T.; Zhang, P.; Bergstrom, D.E. *Nucleic Acids Research* **1997** ***25***, 559-567.
4a. Zaccolo, M.; Williams, D.M.; Brown, D.M.; Gherardi, E. *J. Mol. Biol.* **1996** ***255***, 589-603. 4b. Sala, M., Pezo, V., Pochet, S.; Wain-Hobson, S *Nucleic Acids Research* **1996** ***24***, 3302-3306.
5a. Pochet, S.; D'Ari, R. *Nucleic Acids Res.* **1990** ***23***, 7127-7131. 5b. Pochet, S.; Dugué, L. *Nucleosides and Nucleotides* **1995** ***14***, 1195-1210.
6. Presented in part in XII International Round Table: "Nucleosides and Nucleotides Nucleosides, Nucleotides and their Biological Applications", San Diego, September 15-19 1996.

7. NMR data: Compound **2**: ^{1}H NMR (D_2O) δ: 2.60 (ddd, 1H, H2'); 2.85 (ddd, 1H, H2''); 3.70 (dd, 1H, H5', J = 6.2 and J = 12.3); 3.80 (dd, 1H, H5'', J = 3.9 and J = 12.3); 4.15 (m, 1H, H4'); 4.65 (m, 1H, H3'); 6.41 (dd, 1H, H1', J = 5.1 and J = 6.7); 8.70 (s, 1H, H5). ^{13}C NMR (D_2O) δ: 39.69 (C2'); 62.33 (C5'); 71.47 (C3'); 88.37 (C1' ou C4'); 88.86 (C4' ou C1'); 146.57 (C5); 157.02 (C3); 163.72 ($CONH_2$).Compound **4**: ^{1}H NMR (DMSO-d6) δ: 1.31 (t, 3H, CH_3); 2.35 (m, 1H, H2'); 2.55 (m, 1H, H2''); 3.43 (m, 1H, H5'); 3.54 (m, 1H, H5''); 3.86 (m, 1H, H4'); 4.33 (q, 2H, CH_2); 4.37 (m, 1H, H3'); 4.88 (t, 1H, OH5', J = 5.5); 5.35 (d, 1H, OH3', J = 4.4); 6.28 (t, 1H, H1', J = 6); 8.90 (s, 1H, H5). ^{13}C NMR (DMSO-d6) δ: 14.14 (CH_3); 39.56 (C2'); 61.17 and 61.49 (CH_2 and C5'); 70.09 (C3'); 88.05 and 88.35 (C1' and C4'); 145.29 (C5); 154.15 (C3); 159.48 (COOEt). Phosphoramidites: ^{31}P NMR ($CDCl_3$) δ : 147.10 and 147.18 ppm (**7**); 114.57, 114.64, 147.09, 147.17 (**9**); 147.14 and 146.03 ppm (**8**); 147.06and 147.01 ppm (**13**); 147.28 and 146.99 ppm (**14**).