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Novel 2'-Deoxycytidine Analogues as pH Independent Substitutes of Protonated Cytosines in Triple Helix Forming Oligonucleotides

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Abstract : *The N-acetyl derivatives* ***1a*** *and* ***2a*** *of the aminotriazole and aminoimidazole 2'-deoxynucleosides* ***1*** *and* ***2*** *were synthesized as potential substitutes of protonated 2'-deoxycytidines within triple helix forming oligonucleotides for their recognition of DNA duplexes in a pH independent manner.*

Triple helix forming oligonucleotides (TFO) belonging to the pyrimidine motif, are subject of considerable attention as potential gene regulation agents[1]. Ideally, a TFO recognizes an homopurine strand by means of Hoogsteen hydrogen bonding within the major groove of a DNA duplex target. In these helical complexes, guanine-cytosine (G.C) base pair recognition is specifically achieved by a protonated cytosine (CH^+) (Figure). Consequently, the affinity of TFOs incorporating 2'-deoxycytidines (dC) is pH dependent and considerably decreased at neutral pH in view of the 4.3 value of the pKa of dC. Moreover, in the case of duplexes having a number of contiguous G.C base pairs, the presence of adjacent protonated 2'-deoxycytidines in the third strand induced a high destabilisation of the triplexes by electrostatic charge-charge repulsion[2]. To date, to circumvert this problem many solutions have been proposed which have their own limitations[3].

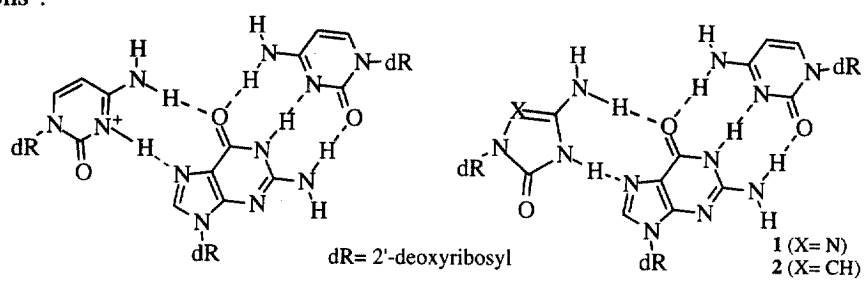

Figure: G.C base pair recognition by five membered ring analogues (*right*) of CH^+ (*left*).

In this laboratory, we propose to design new approaches to improve the binding affinity of TFOs and the stability of their corresponding triplexes[4]. To the best of our knowledge, for the CH^+•G.C triad we noticed that only purines and pyrimidines analogues of dC have been examined. Accordingly, the eventual contribution of the ring size of such analogues in

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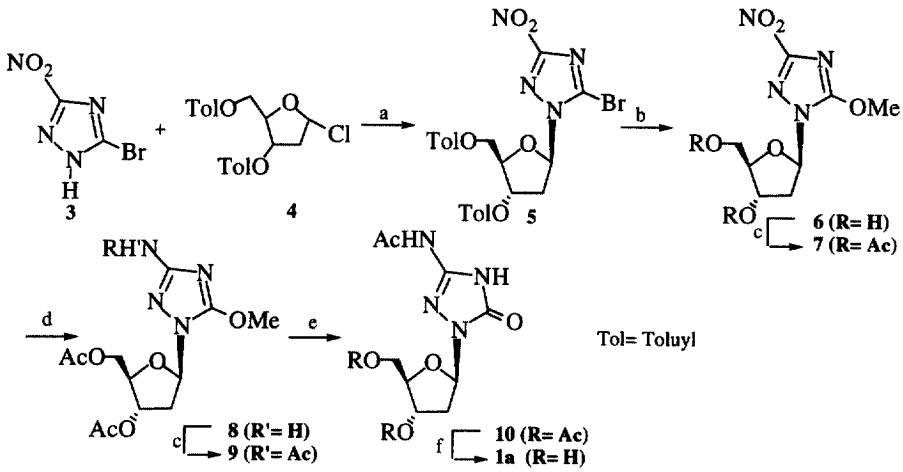
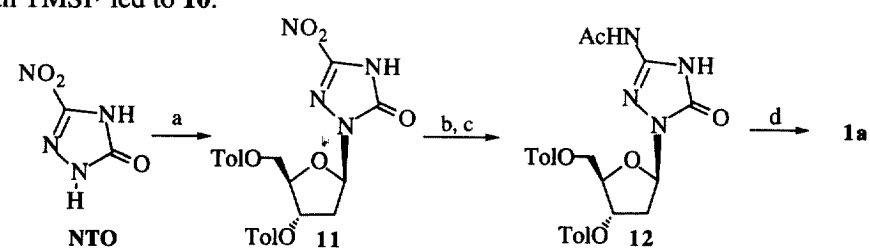
recognition remains to be investigated. Herein we describe the efficient syntheses of 4-aminotriazole and 4-aminoimidazole 2'-deoxynucleosides **1** and **2** which might represent viable substitutes for protonated 2'-deoxycytidines in TFO at physiological pH.



Conditions: a. NaH (1 eq.), **4** (1.2 eq.), MeCN, 60% ; b. MeONa, MeOH, 95%; c. Ac_2O, Py, 98%; d. H_2-Pd/C, MeOH, 97%; e. TMSCl (2eq.), NaI (2 eq.), CH_2Cl_2, 0°C, 87%; f. MeONa (3eq), MeOH, 78%.

Scheme 1. Synthesis of nucleoside **1a** starting from bromo-nitrotriazole

In the case of nucleoside **1** (Scheme 1) the synthesis begins with the glycosylation of 3-bromo-5-nitro-1,2,4-triazole **3** previously described by Robins[5], which in the presence of (α) 2-deoxy-3,5-*o*-ditoluylribosyl chloride **4** yielded the β-nucleoside **5** (60%) together with its N-2-isomer (24%)[6], whose β-stereochemistry at the anomeric position was ascertained by 2D NOE. After some experimentation the following steps were found the most satisfactory to obtain the N-acetylated nucleoside **10**, an immediate precursor of **1a**. Thus, bromine substitution at position **5** was accomplished by a simple treatment with sodium methylate in methanol to give **6** which was acetylated yielding compound **7**. Reduction of the nitro group of this derivative was achieved quantitatively by Pd/C catalyzed hydrogenation to give the aminonucleoside **8** which was acetylated to provide derivative **9**. Subsequently, a treatment of **9** with TMSI[7] led to **10**.

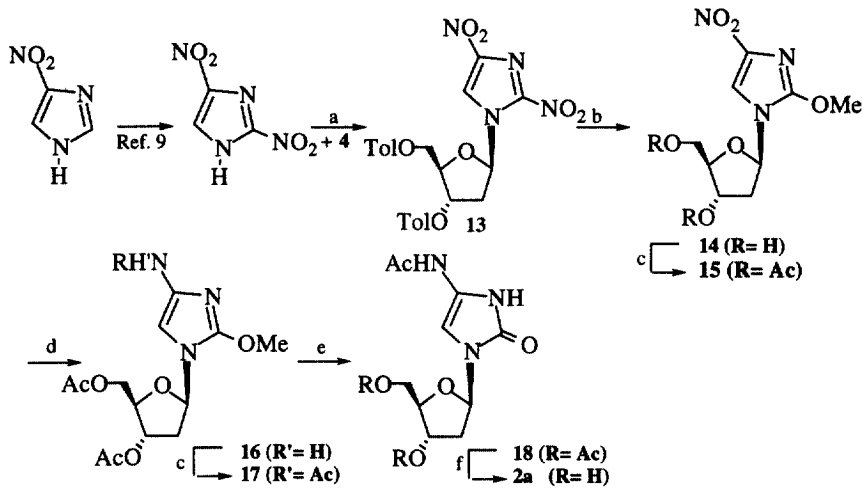


Conditions: a. NaH (1 eq.), **4** (1.2 eq.), MeCN, 67% (β); b. H_2 , Pd/C 50 psi., overnight, 85%. c. Ac_2O, Py, 89%; d. MeONa, MeOH, 92%

Scheme 2. Synthesis of **1a** starting from nitrotriazolone (NTO)

To avoid the successive methanolysis and demethylation steps to introduce the 2-oxo function we decided to develop another new short and convergent synthesis of **1a** (Scheme 2)

starting from nitrotriazolone (NTO)[8]. Glycosylation of NTO led in good yield to nucleoside **11** together with a small amount of its isomer. Catalytic hydrogenation followed by acetylation and removal of the toluyl groups gave the desired aminotriazolone **1a** in good overall yield.



Conditions: a. NaH (1 eq.), **4** (1.2 eq.), MeCN, 89%; b. MeONa, MeOH, 95%; c. Ac_2O, Py, 98%; d. H_2-Pd/C, MeOH, 97%; e. TMSCl (2eq.), NaI (2 eq.), CH_2Cl_2, 0°C, 87%; f. K_2CO_3, MeOH, H_2O, 78%.

Scheme 3: Synthesis of nucleoside **2a** by nucleophilic aromatic substitution of 2-nitro group.

In the imidazole series, a very similar route was followed (Scheme 3). Standard glycosylation of 2,4-dinitroimidazole[9] gave a 89% yield of the separable β nucleoside **13** and its N3-regioisomer in 8/2 ratio. The structural assignment of the two nucleosides was accomplished on the basis of their nmr data. Interestingly, regioselective nucleophilic aromatic substitution (SNAr) of the 2-nitro group of compound **13** led to the deprotected 2-methoxy-4-nitroimidazole nucleoside **14** which is more stable than its parent compound. Success of the methanolysis step requires a very slow addition of sodium methylate to the methanolic solution of the dinitroimidazole nucleoside **13**. Otherwise, the competitive elimination of the electron deficient 2,4-dinitroimidazole takes place instead of 2-nitro substitution. It is noticeable that this undesired reaction can also be observed in the presence of weaker bases such as triethylamine.

Finally, compound **14** was acetylated leading to diacetate **15**. Reduction of the 4-nitro group of the latter was performed under standard Pd/C catalyzed hydrogenation conditions yielding the expected 4-amino derivative **16**. This compound was further N-acetylated providing **17** which readily underwent trimethylsilyl iodide induced demethylation to give the 4-nitro-2-imidazolone **17** in good yield. The O-acetyl groups of derivative **17** could be selectively eliminated to provide the N-acetyl nucleoside **2a**[10,11].

In summary, we have described the efficient synthesis of two new five membered ring heterocyclic analogues of 2'-deoxycytidine. They both exhibit a specific ADD (acceptor-donor-donor) site for a pH independent Hoogsteen mode recognition of guanosine within G.C base pair. Their incorporation in TFO together with the examination of the hybridization properties of the modified oligonucleotides are in progress.

References and Notes

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10. For the incorporation of **1** and **2** into oligonucleotides, **1a** and **2a** need to be 5'-dimethoxytritylated and 3'-phosphitylated.
11. All products gave satisfactory analytical and spectral data. Selected products:
5 ^{1}H NMR ($CDCl_3$, 300 MHz) 2.41 (2s, 6H, 2Me), 2.82 and 3.40 (2m, 2H, H2'), 4.63 (m, 3H, H4' and H5'), 5.81 (m, 1H, H3'), 6.45 (t, 1H, *J*= 6.2 Hz, H1'), 7.33 (2d, 4H, *J*= 8.1 Hz, tol.), 7.91 (2d, 4H, *J*= 8.1 Hz, tol.). ^{13}C NMR 21.7, 36.8, 63.2, 74.3, 84.3, 88.2, 126.3 - 129.7, 131.8, 144.1, 144.6, 165.8, 166.1. MS (IC) *m/z* 546 (MH^+). Calc. for $C_{23}H_{21}BrN_4O_2$ C 50.66, H 3.88, N 10.27; found: C 50.92, H 3.71, N 9.99. **6** ^{1}H NMR ($CDCl_3$, 300 MHz) 2.42 and 2.83 (2m, 2H, H2'), 3.85 (m, 2H, H5'), 4.12 (m, 1H, H4'), 4.33 (s, 3H, OMe), 4.82 (m, 1H, H3'), 6.20 (t, 1H, *J*= 6.2 Hz, H1'). ^{13}C NMR 39.6, 58.6, 62.6, 71.2, 85.1, 88.1, 158.2, 159.0. MS (IC) *m/z* 261 (MH^+). Calc. for $C_8H_{12}N_4O_6$ C 36.93, H 4.65, N 21.53; found: C 36.92, H 4.68, N 21.53. **7** ^{1}H NMR ($CDCl_3$, 300 MHz) 2.13 (2s, 6H, 2Me), 2.62 and 3.10 (2m, 2H, H2'), 4.24 (m, 1H, H4'), 4.35 (s, 3H, OMe), 4.43 (m, 2H, H5'), 5.50 (m, 1H, H3'), 6.21 (t, 1H, *J*= 6.2 Hz, H1'). ^{13}C NMR 20.3, 20.4, 35.4, 59.5, 63.0, 74.1, 83.0, 84.8, 158.0, 159.0, 170.0, 170.2. MS (IC) *m/z* 344 (MH^+). **8** ^{1}H NMR ($CDCl_3$, 300 MHz) 2.12 (2s, 6H, 2Me), 2.30 and 2.96 (2m, 2H, H2'), 4.02 (s, 3H, OMe), 4.10-4.40 (m, 3H, H4' and H5'), 4.42 (br s, 2H, NH_2), 5.45 (m, 1H, H3'), 6.03 (t, 1H, *J*= 6.0 Hz, H1'). ^{13}C NMR 20.6, 20.7, 34.9, 57.9, 63.9, 74.6, 81.6, 83.0, 158.6, 159.2, 170.2, 170.6. MS (IC) *m/z* 315 (MH^+). **9** ^{1}H NMR ($CDCl_3$, 300 MHz) 2.14 (2s, 6H, 2Me), 2.32 (s, 3H, NHAc), 2.40 and 3.05 (2m, 2H, H2'), 4.13 (s, 3H, OMe), 4.10-4.40 (m, 3H, H4' and H5'), 5.43 (m, 1H, H3'), 6.20 (t, 1H, *J*= 6.0 Hz, H1'). ^{13}C NMR 20.6, 20.7, 25.4, 35.2, 58.5, 63.4, 74.4, 85.5, 83.8, 152.5, 159.4, 169.9, 171.5. MS (IC) *m/z* 357 (MH^+). **10** ^{1}H NMR ($CDCl_3$, 300 MHz) 2.10 (2s, 6H, 2Me), 2.20 and 2.85 (2m, 2H, H2'), 2.53 (s, 3H, NHAc), 4.12-4.40 (m, 3H, H4' and H5'), 5.34 (m, 1H, H3'), 6.10 (t, 1H, *J*= 6.0 Hz, H1'). ^{13}C NMR 20.8, 20.9, 23.7, 34.2, 64.2, 74.4, 81.5, 82.9, 145.2, 149.4, 170.4, 170.8, 171.4. MS (IC) *m/z* 343 (MH^+). **1a** ^{1}H NMR ($CDCl_3$ - CD_3OD, 300 MH) 2.12 (s, 3H, NHAc), 2.41 and 2.86 (2m, 2H, H2'), 3.70-4.20 (m, 3H, H4' and H5'), 4.46 (m, 1H, H3'), 6.0 (t, 1H, *J*= 6.1 Hz, H1'). ^{13}C NMR 23.5, 37.2, 61.2, 71.1, 82.1, 86.7, 158.0, 159.0, 169.2. MS (IE) *m/z* 258 (M^+). **11** ^{1}H NMR ($CDCl_3$, 300 MHz) 2.22 (2s, 6H, 2Me), 2.51 and 3.33 (2m, 2H, H2'), 4.62 (m, 3H, H4' and H5'), 5.81 (m, 1H, H3'), 6.59 (t, 1H, *J*= 6.1 Hz, H1'), 7.30-7.92 (4d, 8H, *J*= 8.1 Hz, tol.). ^{13}C NMR 21.7, 34.6, 63.8, 74.6, 84.9, 86.3, 144.0, 144.7, 152.0, 166.0, 166.2. MS (IC) *m/z* 353 (MH^+). **12** ^{1}H NMR ($CDCl_3$, 300 MHz) 2.40 (2s, 6H, 2Me), 2.51 (s, 3H, Ac), 2.52 and 2.84 (2m, 2H, H2'), 4.40-4.93 (m, 3H, H4' and H5'), 5.12 (m, 1H, H3'), 6.10 (t, 1H, *J*= 6.1 Hz, H1'), 7.21-7.93 (4d, 8H, *J*= 8.2 Hz, tol.). ^{13}C NMR 21.7, 23.5, 35.2, 63.9, 74.2, 82.8, 83.3, 126.2 - 129.8, 144.3, 144.7, 150.2, 165.7, 166.3. MS (IC) *m/z* 495 (MH^+). **13** ^{1}H NMR ($CDCl_3$, 300 MHz) 2.40 (2s, 6H, 2Me), 2.51 and 3.22 (2m, 2H, H2'), 4.82 (m, 3H, H4' and H5'), 5.71 (m, 1H, H3'), 6.80 (t, 1H, *J*= 6.2 Hz, H1'), 7.22-7.94 (4d, 8H, *J*=8 Hz, tol.), 8.41 (s, 1H, H5). ^{13}C NMR 39.1, 63.3, 74.0, 85.2, 91.0, 120.1, 126.1, 129.5, 129.6, 129.9, 166.2. MS (IC) *m/z* 511 (MH^+). **14** ^{1}H NMR (DMSO d_6, 300 MHz) 2.42 and 2.81 (2m, 2H, H2'), 3.51 (m, 2H, H5'), 3.82 (m, 1H, H4'), 4.03 (s, 3H, OMe), 4.91 (m, 1H, H3'), 6.02 (t, 1H, J= 6.1 Hz, H1'), 8.30 (s, 1H, H5). ^{13}C NMR 39.8, 57.6, 61.1, 70.0, 83.7, 87.9, 116.3, 142.2, 150.1. MS (IC) *m/z* 260 (MH^+). **2a** ^{1}H NMR (CD_3OD, 300 MHz) 2.21 (s, 3H, NHAc), 2.30 and 2.52 (2m, 2H, H2'), 3.60-4.10 (m, 3H, H4' and H5'), 4.62 (m, 1H, H3'), 5.96 (t, 1H, J= 6.1 Hz, H1'), 8.23 (s, 1H, H5). ^{13}C NMR 24.1, 37.5, 59.9, 71.1, 81.4, 85.9, 115.7, 141.0, 152.1. MS (IE) *m/z* 257 (M^+).