Synthesis of Pyridine-stretched 2′-Deoxynucleosides

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Abstract: Synthesis of novel pyridine-stretched nucleoside (PSN) analogues of adenine (strA) (**1**), 2,6-diaminopurine (strD) (**15**) and hypoxanthine (strH) (**17**) from 4(5)-nitroimidazole has been achieved. Glycosylation of 4(5)-nitroimidazole was optimized to give consistently good yields (>70%) of the desired analytically pure 5-nitro-1′-β isomer **8** which on hydrogenation, C-addition of ethoxymethylene malononitrile (EMMN) and cyclisation provides the key intermediate **14** for PSN synthesis.

Key words: 2′-deoxynucleosides, 5-aminoimidazoles, DNA, *lin*-pyridoadenosine, glycosylation, nucleoside analogues

To determine the effect of extended purine base structures on the stability of triplex-forming oligonucleotides (TFOs) targeted to DNA, we identified a series of pyridine-stretched nucleosides (PSNs), including the 2′-deoxyadenosine analogue **1**, for potentially advantageous incorporation into TFOs. PSNs are linear tricyclic analogues of the naturally occurring purine nucleosides with a central pyridine ring inserted between the pyrimidine and imidazole rings.[1] Benzene-stretched nucleoside analogues, such as *lin*-benzoadenosine **2** (Figure 1), have previously been described by Leonard and co-workers[2] but their difficult synthesis hinders access to the necessary quantities for oligomerization and study of DNA molecular recognition properties. Here we describe the synthesis of three pyridine-stretched 2′-deoxynucleosides: stretched 2′-deoxyadenine **1** (strA), stretched 2′-deoxydiaminopurine **15** (strD) and stretched 2′-deoxyhypoxanthine **17** (strH).

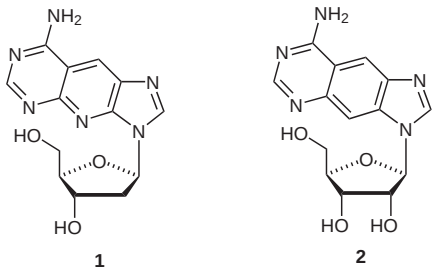

Figure 1

The development of TFOs for controlling gene expression at the transcription level continues to attract attention with much synthetic effort employed in the development of novel base analogues for stronger triple helix formation with targeted DNA.[3] Replacement of thymidine and cytosine by tricyclic base analogues has been shown to either increase or decrease the stability of DNA duplexes with the outcome very much dependant upon the precise structural changes made. Replacement of thymidine by the extended thymidine analogues benzo[*f*]quinazoline-2,4-dione (linear) or benzo[*g*]quinazoline-2,4-dione (crescent-shaped), causes duplex destabilization[4] whereas replacement of cytosine by linear phenothiazine and phenoxazine tricyclic derivatives enhances duplex stability.[5]

To prepare the novel 2′-deoxynucleosides **1**, **15** and **17** we selected as starting material the well known 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-*erythro*-pentofuranosyl chloride **7**, which is prepared as the crystalline α-anomer in three steps from 2-deoxyribose.[6] In previous glycosylations to prepare ribonucleoside analogues, we employed the silver salt of ambident nucleophile 4(5)-nitroimidazole and, aided by participation of the neighbouring 2′-substituent, obtained only β-anomers with the required 5-nitro-1′-β regioisomer predominating.[1] Using the same conditions, we carried out glycosylation of the silver salt **3** with the 1′-chloro-2′-deoxysugar **7** in xylene to yield a mixture of β-anomers in which the required 5-nitro-regioisomer **8** and the 4-nitro-regioisomer **9** were in a ratio of 1:2 (Scheme 1). The required isomer **8**[7] was isolated in pure crystalline form by column chromatography to produce useable quantities of material. However, the selectivity of this glycosylation required improvement. The outcome of glycosylations using the chlorosugar **7** is strongly influenced by choice of solvent, nucleophile and concentration.[8] We repeated the glycosylation of the silver salt **3** in acetonitrile to yield a near even distribution of all four possible isomers (**8**–**11**, Scheme 1). Use of the lithium salt **4** under these reaction conditions resulted in an increased yield of the α-anomers (**10** and **11**) at the expense of a lower yield of each β-anomer (**8** and **9**). Bergstrom and co-workers[9] reported that under standard sodium salt glycosylation conditions,[10] where the sodium salt **5** is generated in situ using sodium hydride before addition of chlorosugar **7**, β-anomers (**8** and **9**) are isolable in a 1:2 ratio. By using the preformed sodium salt **5**, we slightly improved the yield and anomeric ratio, (1:1.5) and detected the presence of small amounts of the two α-anomers (**10** and **11**).

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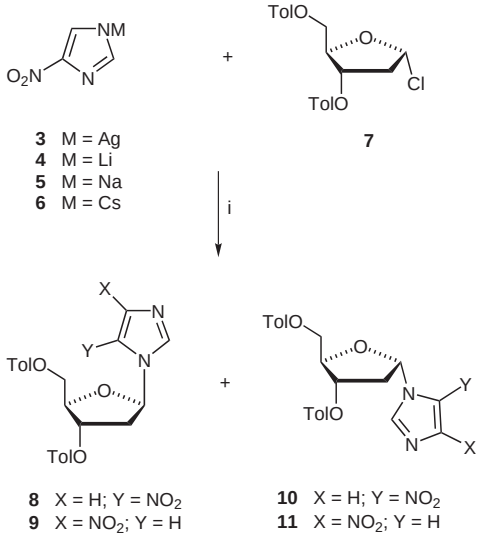


Scheme 1 *Reagents and conditions*: i, THF, 62 °C, 3 h.

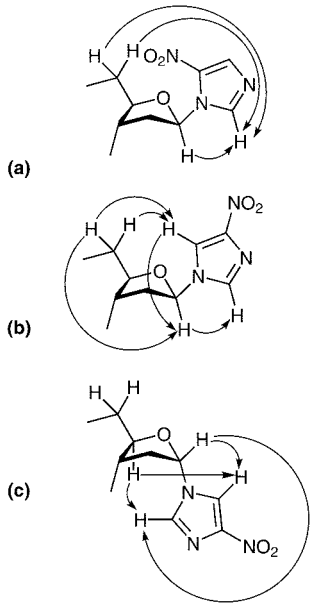


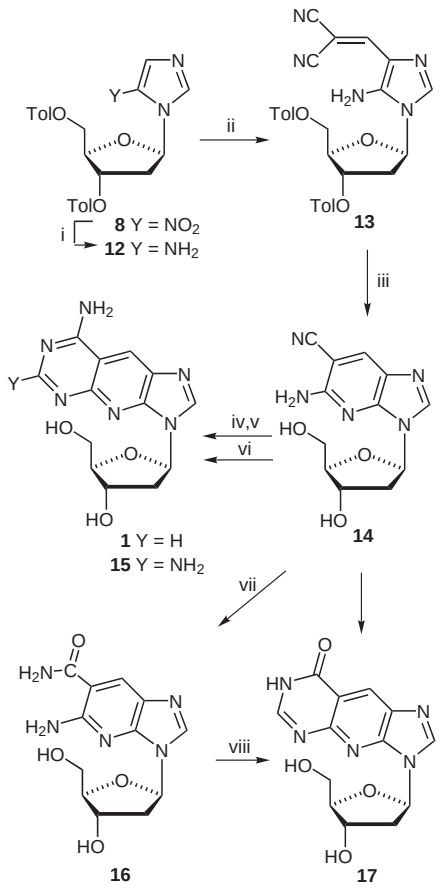
Figure 2 Strong NOE enhancements from NOSEY spectra: (a) compound **8**; (b) compound **9**; (c) compound **11**.

Glycosylation using α-chlorosugar **7** probably proceeds by an S_N2 Walden inversion process at C-1′ to give the β-product. Formation of the α-nucleosides by a similar process can be explained if chlorosugar **7** first anomerises. This anomerisation occurs readily with solvents of higher dielectric constant and is further enhanced by the presence of lithium, silver and to a lesser extent sodium ions.[8,11] We observed by ^{1}H NMR that over three hours (the time for completion of these glycosylation reactions) the chlorosugar **7** in d_3-acetonitrile underwent 40% inversion to the more reactive[11] β-anomer, compared with less than 5% anomerisation in d_8-THF. We expected, therefore, that replacement of acetonitrile by less polar THF would favour formation of β-anomers (**8** and **9**) over α-anomers (**10** and **11**) and this led us to investigate this solvent in combination with the cesium salt **6**. We reasoned that use of the cesium salt **6** would also lead to an improvement of the β/α ratio because Cs^+ is less likely to co-ordinate to the 2′-chloro substituent and enhance anomerisation of the chlorosugar **7**. Glysosylation of cesium salt **6** with chlorosugar **7** for three hours at 62 °C in THF gave a 71% isolated yield of the required pure 5-nitro-1′-β-isomer **8**. Clearly, use of the cesium salt not only improves the anomeric ratio (β/α) but also improves the regioselectivity (**8**/**9**) of the reaction. This preparation is amenable to scale-up giving gram quantities of compound **8** in consistent yields of at least 70%.

The identities of all four isomers (**8**–**11**) were established by ^{1}H and ^{13}C NMR analysis with 5-nitro-1′-β (triplet, δ = 6.74 ppm) and 5-nitro-1′-α (doublet, δ = 6.80 ppm) anomeric proton signals being downfield relative to those of the 4-nitro-1′-β (triplet, δ = 6.15ppm) and 4-nitro-1′-α (doublet, δ = 6.25 ppm) signals. These structural assignments were confirmed by NOESY. The strong NOE enhancements are shown in Figure 2.

Catalytic reduction (5% Pd/C–H_2–THF) of the 5-nitroimidazole **8** gave the amine **12** which was immediately reacted with ethoxymethylene malononitrile (EMMN). In accord with our previous work with 5-aminoimidazoles, the EMMN gave exclusively the *C*-addition-elimination product **13**,[12] which is associated with a single imidazole ring proton (δ = 7.83 ppm) and a primary amine signal (δ = 7.72 ppm). Treatment of this 2,2-dicyanovinyl derivative **13** with methanolic NaOH under reflux (15 min) resulted in simultaneous cyclisation and deprotection giving the imidazo[4,5-*b*]pyridine derivative **14** (84%).[13] The structure of nitrile **14** is fully supported by its spectroscopic properties. This product (**14**), formed in three isolation steps from the 4(5)-nitroimidazole cesium salt **6** (Scheme 1), is a key common intermediate for the preparation of PSNs.

The imidazo[4,5-*b*]pyridine **14** was converted to strA (**1**) without isolation of intermediates by the following procedure. A suspension of compound **14** in excess diethoxymethyl acetate was heated under reflux (2 h). Evaporation gave a light brown oil (*O*-acetylimidate), which was immediately treated with methanolic ammonia (16 h). Removal of the solvent gave a residue (formamidine) that was treated with aqueous HOAc (16 h) and neutralized to give a crystalline product. This was recrystallised from H_2O and identified as strA (**1**, 47%).[14] The nitrile **14** was converted to strD (**15**) in a single step. Guanidine hydrochloride was converted to the free base by stirring with NaOMe in MeOH. One equivalent of nitrile **14** was added and the mixture heated in a steel bomb at 145 °C for 42 h. The solid product was filtered off and washed with H_2O and then with EtOH to yield strD (**15**, 45%).[15] Finally, the nitrile **14** was converted to strH (**17**, Scheme 2). Hydrolysis using H_2O_2/NH_4OH (20 °C, 40 min) gave the amide **16**, which was not isolated but, after evaporation, was immediately cyclised using ethyl formate and ethanolic NaOEt at reflux temperature (2 h). The residue crystallized from H_2O (adjusted to pH 6 using 2 M aq HCl) and



Scheme 2 *Reagents and conditions*: i, H_2, 5% Pd/C, THF, 2 h; ii, $EtOCH{=}C(CN)_2$; iii, MeOH–NaOH (aq), 90 °C, 15 min; iv, $MeCO_2CH(OEt)_2$, reflux, 2 h; v, MeOH–NH_3; vi, $HN{=}C(NH_2)_2$, 145 °C, 42 h; vii, H_2O_2/NH_4OH, 20 °C, 40 min; viii, HCO_2Et–EtOH–NaOEt, reflux, 2 h.

was identified as strH (**17**, 44%).[16] All three pyridine-stretched 2′-deoxyribonucleosides (**1**, **15** and **17**) were fully characterized by their spectroscopic properties which are entirely consistent with the proposed stereo- and regio-chemistry.[14–16]

In conclusion, we have described a viable synthetic route to 'pyridine-stretched' nucleoside analogues via the 1′-β-2′-deoxy-5-nitroimidazole derivative **8**. For 2′-deoxy nucleoside analogues a substituent at position 2′ cannot be used to control the stereochemistry at the anomeric position and we have therefore investigated reaction conditions with the objective of optimising formation of the desired stereoisomer **8**. We have found that the imidazole **8** can be obtained selectively in >70% yield by using the cesium salt of 4(5)-nitroimidazole and THF as solvent. This product **8** is then converted in two isolation steps to the key intermediate **14**, which is then readily transformed into tricyclic 2′-deoxynucleoside analogues.

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(7) Compound **8** (70%): Mp 162 °C (colourless solid). IR (KBr): ν_{max} = 752, 1092, 1111, 1280, 1374, 1466, 1528, 1723, 2938 cm^{-1}. ^1H NMR ($CDCl_3$): δ = 2.42 (3 H, s, ArCH_3), 2.46 (3 H, s, ArCH_3), 2.52 (1 H, m, 2′-C*H*), 3.12 (1 H, m, 2′-C*H*), 4.74 (3 H, m, 5′-CH_2 and 4′-C*H*), 5.64 (1 H, m, 3′-C*H*), 6.74 (1 H, t, 1′-C*H*), 7.24 (2 H, d, Ar*H*), 7.30 (2 H, d, Ar*H*), 7.84 (2 H, d, Ar*H*), 7.97 (2 H, d, Ar*H*), 8.05 [1 H, s, imidazole(4)-*H*], 8.11 [1 H, s, imidazole(2)-*H*]. ^{13}C NMR ($CDCl_3$): δ = 21.70 (q, ArCH_3), 21.77 (q, ArCH_3), 40.86 (t, 2′-CH_2), 63.60 (t, 5′-CH_2), 74.14 (d, 4′-*C*H), 83.94 (d, 3′-*C*H), 88.99 (d, 1′-*C*H), 126.04 [s, Ar(4)-*C*], 126.18 [s, Ar(4)-*C*], 129.32 (d, Ar-*C*H), 129.39 (d, Ar-*C*H), 129.55 (d, Ar-*C*H), 129.81 (d, Ar-*C*H), 134.33 [d, imidazole(4)-*C*H], 138.05 [v weak s, imidazole(5)-*C*], 138.13 [d, imidazole(2)-*C*H], 144.50 [s, Ar(1)-*C*], 144.70 [s, Ar(1)-*C*], 165.92 (s, *C*=O), 166.07 (s, *C*=O). MS (EI): *m/z* (%) = 465 (2) [M^+], 320 (1), 216 (28), 136 (28), 119 (100), 91 (53), 81 (93), 65 (18), 53 (14), 39 (13), 28 (13). Anal. Calcd for $C_{24}H_{23}N_3O_7$: C, 61.9%; H, 4.98%; N, 9.0%. Found: C, 61.7%; H, 4.89%; N, 8.8%.
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(12) Compound **13** (43%): Mp 194–196 °C (yellow needles). IR (KBr): ν_{max} = 752, 1112, 1266, 1283, 1354, 1540, 1586, 1715, 2218, 2924, 3348 cm^{-1}. ^1H NMR (d_6-DMSO): δ = 2.39 (3 H, s, ArCH_3), 2.41 (3 H, s, ArCH_3), 2.72 (1 H, m, 2′-C*H*), 2.86 (1 H, m, 2′-C*H*), 4.53 (3 H, br s, 5′-CH_2 and 4′-C*H*), 5.63 (1 H, d, 3′-C*H*), 6.09 (1 H, dd, 1′-C*H*), 7.34 (2 H, d, Ar*H*), 7.38 (2 H, d, Ar*H*), 7.72 (2 H, br s, NH_2), 7.79 (1 H, s, C=C*H*), 7.83 [1 H, s, imidazole(2)-*H*], 7.86 (2 H, d, Ar*H*), 7.95 (2 H, d, Ar*H*). ^{13}C NMR (d_6-DMSO): δ = 21.14 (q, ArCH_3), 21.17 (q, ArCH_3), 35.88 (t, 2′-CH_2), 58.63 [s, $C(CN)_2$], 63.96 (t, 5′-CH_2), 74.72 (d, 4′-*C*H), 81.69 (d, 3′-*C*H), 82.81 (d, 1′-*C*H), 116.20 [s, imidazole(4)-*C*], 117.99 (s, *C*N), 118.56 (s, *C*N), 126.43 [s, 2 × Ar(4)-*C*], 129.26 (d, 2 × Ar-*C*H), 129.32 (d, Ar-*C*H), 129.47 (d, Ar-*C*H), 134.13 [d, imidazole(2)-*C*H], 143.53 (d, C=*C*H), 143.84 [s, Ar(1)-*C*], 144.08 [s, Ar(1)-*C*], 150.02 [s, imidazole(5)-*C*], 165.12 (s, *C*=O), 165.40 (s, *C*=O). MS (EI): *m/z* (%) = 511 (7) [M^+], 353 (7), 159 (41), 119 (68), 91 (23), 81 (100).
(13) Compound **14** (84%): Mp 162 °C (colourless needles). IR (KBr): ν_{max} = 940, 1099, 1432, 1576, 1630, 2216, 2923, 3223, 3337 cm^{-1}. ^1H NMR (d_6-DMSO): δ = 2.23 (1 H, m, 2′-C*H*), 2.59 (1 H, m, 2′-C*H*), 3.52 (2 H, m, 5′-CH_2), 3.83 (1 H, d, 4′-C*H*), 4.37 (1 H, s, 3′-C*H*), 4.96 (1 H, t, 5′-O*H*), 5.32 (1 H, d, 3′-O*H*), 6.29 (1 H, t, 1′-C*H*), 6.79 (2 H, br s, NH_2), 8.24 (1 H, s, pyridine-*H*), 8.42 [1 H, s, imidazole(2)-*H*]. ^{13}C NMR (d_6-DMSO): δ = 39.51 (t, 2′-CH_2), 61.89 (t, 5′-CH_2), 70.99 (d, 4′-*C*H), 82.71 (d, 3′-*C*H), 86.26 (s, *C*.CN), 87.89 (d, 1′-CH), 118.02 (s, *C*N), 127.11 (s, *C*), 134.37 [d, imidazole(2)-*C*H], 142.29 (d, pyridine-*C*H), 148.96 (s, *C*), 157.84 (s, *C*). MS (EI): *m/z* (%) = 275 (84) [M^+], 186 (100),

160 (10 0), 159 (100), 132 (35), 117 (63), 99 (32), 73 (50), 45 (39), 43 (36), 28 (26). HRMS (EI): *m/z* = calcd for $C_{12}H_{13}N_5O_3$: 275.1018. Found: 275.1008 [M^+].

(14) Compound **1** (47%). Mp >280 °C (colourless needles). IR (KBr): ν_{max} = 812, 916, 1091, 1419, 1507, 1577, 1685, 3086 cm^{-1}. ^1H NMR (d_6-DMSO): δ = 2.36 (1 H, m, 2′-C*H*), 2.83 (1 H, m, 2′-C*H*), 3.63 (2 H, m, 5′-CH_2), 3.93 (1 H, m, 4′-C*H*), 4.49 (1 H, s, 3′-C*H*), 5.15 (1 H, t, 5′-O*H*), 5.40 (1 H, d, 3′-O*H*), 6.74 (1 H, t, 1′-C*H*), 8.09 (2 H, br s, NH_2), 8.51 (1 H, s, 2-*H*), 8.92 (1 H, s, 6-*H*), 9.07 (1 H, s, 9-*H*). ^{13}C NMR (d_6-DMSO): δ = 39.44 (t, 2′-*C*H_2), 61.95 (t, 5′-*C*H_2), 71.06 (d, 4′-*C*H), 83.81 (d, 3′-*C*H), 88.19 (d, 1′-*C*H), 106.43 (s, *C*), 123.80 (d, *C*H), 134.68 (s, *C*), 148.25 (d, *C*H), 151.01 (s, *C*), 155.73 (s, *C*), 157.87 (d, *C*H), 164.11 (s, *C*). MS (FAB): *m/z* (%) = 303 (53) [M + H), 187 (31), 165 (30), 152 (46), 124 (36), 120 (46), 115 (35), 107 (100), 105 (43). HRMS-FAB: *m/z* calcd for $C_{13}H_{15}N_6O_3$: 303.1206. Found: 303.1209 [M^+ + H].

(15) Compound **15** (45%). Mp >280 °C (cream solid). IR (KBr): ν_{max} = 803, 1057, 1356, 1470, 1621, 1641, 2887, 3110, 3183, 3318, 3434, 3481 cm^{-1}. ^1H NMR (d_6-DMSO): δ = 2.23 (1 H, m, 2′-C*H*), 2.81 (1 H, m, 2′-C*H*), 3.61 (2 H, m, 5′-CH_2), 3.88 (1 H, m, 4′-C*H*), 4.44 (1 H, m, 3′-C*H*), 5.17 (1 H, t, 5′-O*H*), 5.37 (1 H, d, 3′-O*H*), 6.19 (2 H, br s, NH_2), 6.42 (1 H, t, 1′-C*H*), 7.44 (2 H, br s, NH_2), 8.64 (1 H, s, 6-*H*), 8.83 (1 H, s, 9-*H*). ^{13}C NMR (d_6-DMSO): δ = 39.17 (t, 2′-*C*H_2), 62.08 (t, 5′-*C*H_2), 71.17 (d, 4′-*C*H), 83.84 (d, 3′-*C*H), 88.01 (d, 1′-*C*H), 102.19 (s, *C*), 123.83 (d, *C*H), 131.33 (s, *C*), 145.35 (d, *C*H), 150.76 (s, *C*), 158.27 (d, *C*H), 162.78 (s, *C*), 164.21 (s, *C*). MS (FAB): *m/z* (%) = 318 (91) [M + H], 202 (100), 141 (15), 121 (9). HRMS-FAB: *m/z* calcd for $C_{13}H_{15}N_7NaO_3$: 340.1134. Found: 340.1129 [M^+ + Na].

(16) Compound **17** (44%). Mp >280 °C (cream solid). IR (KBr): ν_{max} = 807, 1086, 1235, 1392, 1605, 1686, 2925, 3214, 3504 cm^{-1}. ^1H NMR (d_6-DMSO) δ = 2.34 (1 H, m, 2′-C*H*), 2.81 (1 H, m, 2′-C*H*), 3.62 (2 H, m, 5′-CH_2), 3.93 (1 H, d, 4′-C*H*), 4.49 (1 H, br s, 3′-C*H*), 5.10 (1 H, t, 5′-O*H*), 5.41 (1 H, d, 3′-O*H*), 6.58 (1 H, t, 1′-C*H*), 8.32 (1 H, s, 2-*H*), 8.75 (1 H, s, 6-*H*), 8.95 (1 H, s, 9-*H*). ^{13}C NMR (d_6-DMSO): δ = 45.58 (t, 2′-*C*H_2), 67.94 (t, 5′-*C*H_2), 77.05 (d, 4′-*C*H), 89.99 (d, 3′-*C*H), 94.28 (d, 1′-*C*H), 120.59 (s, *C*), 132.26 (d, *C*H), 140.99 (s, *C*), 153.83 (d, *C*H), 156.48 (s, *C*), 161.15 (s, *C*), 168.41 (s, *C*=O). MS (FAB): *m/z* (%) = 304 (11) [M + H], 216 (16), 119 (43), 114 (22), 87 (41), 82 (100). HRMS-FAB: *m/z* calcd for $C_{13}H_{13}N_5O_4$: 304.1046. Found: 304.1041 [M + H].