Model studies towards the synthesis of 4′-azaerythrofuranosyl-adenines as analogues of the antiviral drug 2′,3′-dideoxyadenosine (ddA)[1]

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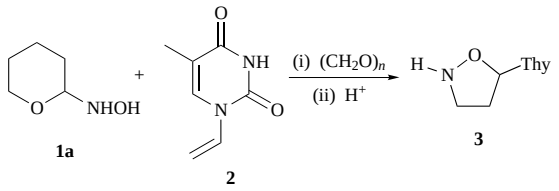
The [3 + 2] dipolar cycloaddition of nitrones to *N*-9-vinyladenine provides a regioselective and stereoselective method for the obtainment of 4′-aza-analogues of 2′,3′-dideoxyadenosine. The latter may act as antiviral agents by analogy with the behaviour *in vitro* of the corresponding thymine analogue. The formation of the unsubstituted isoxazolidino derivative requires the protection of the 6-NH_2 function of the dipolarophile, which is not necessary when preformed nitrones are used. The obtainment of a specific enantiomer has been exploited by enzyme-catalysed hydrolysis of an ester function introduced into the isoxazolidino nucleus.

[3 + 2] Dipolar cycloadditions of nitrones to suitable dipolarophiles are becoming of increasing interest in the synthesis of modified nucleosides as potential inhibitors of reverse transcriptase in AIDS therapy.[2–5]

Results and discussion

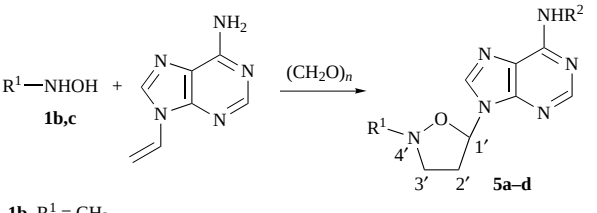
The nitrogen atom of the azatetrahydrofuranose ring of compound **3** (Scheme 1) replaces the 4′-chiral carbon of the dideoxyribose analogue, thus providing the system with more conformational degrees of freedom. In a previous paper[5] we have shown that 2′,3′-dideoxy-4′-azaerythrofuranosylthymine (AdT, **3**), can be prepared in good yields by direct cycloaddition of the tetrahydropyranyl-protected nitrone, generated *in situ* from compound **1a**, to vinylthymine **2** (Scheme 1). The



1a **2** **3**

Scheme 1

isoxazolidino nucleoside thus prepared has shown interesting biological activity *in vitro*.[6] The synergic action of 'drug cocktails' seems to be a convenient therapeutic approach to delaying the devastating action of AIDS.[7] It is therefore important to exploit the synthesis of adenine furanosides of type **5** (Scheme 2) to be monitored for their biological activity as analogues of dideoxyadenosine (ddA), whose antiviral properties are known.[8] The 1,3-dipolar cycloaddition approach represents the method of choice when the dipolarophiles are readily available and the dipoles can be obtained *in situ* by means of paraformaldehyde. The latter is known to react with DNA and nucleosides[9] but no modification of the nucleobase was observed when compound **3** (Scheme 1) was prepared. When hydroxylamines **1b** and **1c** were allowed to react with *N*-9-vinyladenine **4a**[10] in the presence of paraformaldehyde (Scheme 2) complex mixtures of products were obtained which were difficult to separate and characterize. Compounds **5a** and **5c** were isolated with 15 and 5% yield, respectively, whereas the presence of compounds **5b** and **5d** was confirmed by FAB-MS/MS on the crude mixture and in the case of compound **5d** by its isolation as a crystalline product.



1b $R^1 = CH_3$
1c $R^1 = PhCH_2$

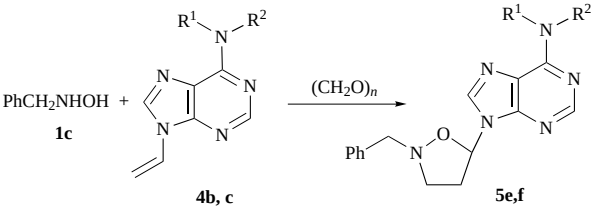
5a $R^1 = CH_3$, $R^2 = H$
5b $R^1 = CH_3$, $R^2 = CH_2OH$
5c $R^1 = PhCH_2$, $R^2 = H$
5d $R^1 = PhCH_2$, $R^2 = CH_2OH$

Scheme 2

The structures of products **5b** and **5d** were proposed on the ground of spectroscopic evidence with reference to the known hydroxymethylation site of *N*-9 substituted adenines.[9] The interactions of purine bases with formaldehyde can lead also to dihydroxymethylated derivatives as experimentally verified by FAB-MS on the crude mixture; therefore, it is reasonable to suggest that the complexity of the cycloaddition process reported in Scheme 2 is due to the competitive reaction paths available to paraformaldehyde in the adopted experimental conditions.

Two *N*-6-protected 9-vinyladenines have recently become available[11] which are suitable for the exploitation of the cycloaddition process when the exocyclic amino function of adenine is masked. The base-labile protection widely employed in DNA chemistry[12] proved to be unsuccessful since compound **5e** was obtained with 7% isolated yield, and the reaction mixture clearly indicated interaction between the hydroxylamine and the phthalimido group of **4b** (Scheme 3).

Conversely, compound **4c** afforded a clean process allowing



4b, c **5e,f**

4b, 5e R^1R^2 = phthaloyl
4c, 5f R^1 = H, R^2 = dimethoxytrityl

Scheme 3

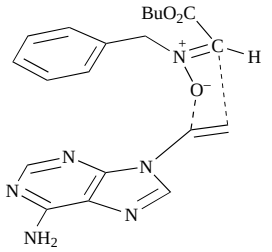
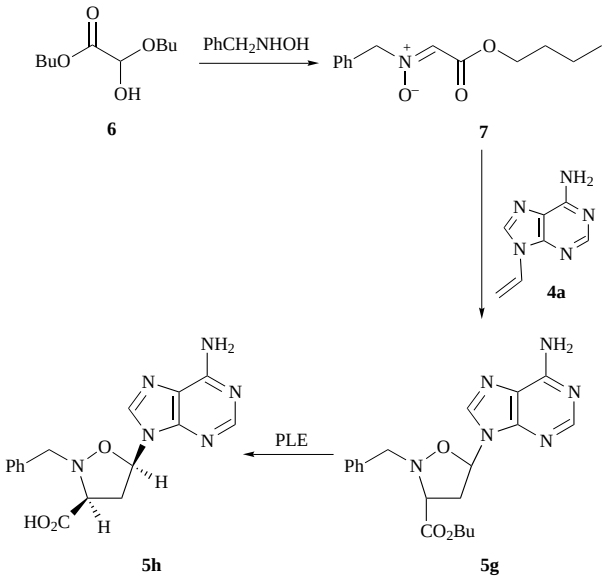


Fig. 1 Proposed transition-state geometry for the *endo* approach between vinyladenine **4a** and nitrone **7**

the isolation of product **5f** in 77% yield. The protected aza-furanoside **5f** was then smoothly converted by mild acid treatment into the unprotected derivative **5c** (Scheme 2) in 84% isolated yield. The role of formaldehyde in driving the side-reactions previously reported is further supported by the smooth formation of the 2′-deoxyadenosine derivatives **5g** and **5h** from stabile nitrones (Scheme 4).



Scheme 4

In this case, in fact, the dipole **7** was prepared in quantitative yield in a 6.3:1 *E*:*Z* isomeric mixture following a literature procedure from the hemiacetal **6**,[13] therefore paraformaldehyde was not present in the reaction milieu of the cycloaddition process.

Product **5g** separates, after a time, from the crude reaction mixture as a racemate. The reaction was regiospecific, as expected for an electron-rich monosubstituted dipolarophile[14] such as compound **4a**. ^{1}H NMR analysis has shown that the relative configuration of the nucleobase and the ester function on the 3′ position is *cis*. The observed stereoselectivity is likely to be due to a preferred *endo* approach between vinyladenine and the *E*-isomer of the dipole, the most reactive under the adopted experimental conditions,[15] driven by stacking interactions which lower the transition-state energy for the formation of product **5g** (Fig. 1).

The ester function was chosen as a handle for the resolution of the racemate with pig liver esterase (PLE).[16] The derived acid **5h** (Scheme 4) was isolated and characterized and its stereochemistry was assigned by extensive ^{1}H NMR decoupling experiments.

The data previously discussed show different approaches for the obtainment of aza analogues of adenine nucleosides. They demonstrate that the cycloaddition approach is the shortest path to isoxazolidinyladenines, provided that suitable experimental conditions, such as the protection of the amino function of substrate **4a**, are chosen when 2′,3′-dideoxy analogues are formed.

Experimental

Mp were determined on a Kofler hot-stage apparatus and are uncorrected. ^{1}H NMR spectra were measured at 300 MHz on a Bruker AC 300 spectrometer as dilute solutions in [$^2H_6$]DMSO. The chemical-shift values (δ) are expressed in ppm relative to an internal tetramethylsilane standard. All coupling constants *J* are reported in Hz. Mass spectra were recorded on a Fisons Vacuum Generators ZAB-2F spectrometer, from 2 mm^3 of 3-nitrobenzyl alcohol/sample mulls, by fast-atom bombardment (FAB$^+$MS), with a neutral xenon beam operating at 8 keV and a total current of ~ 10 μA. Optical-activity measurements were performed on 1 cm^3 solution of the sample in DMSO, using an Atago Polax-D polarimeter equipped with an optical-length cell of 1 dm and a volume of 1 cm^3. Reaction mixtures and the purity of compounds were monitored by TLC using Merck silica gel 60-F_{254} pre-coated aluminium plates. Short-column flash chromatography (SCFC) was performed on Merck Kieselgel 60H without gypsum. The 1,3-dipolar cycloadditions were carried out under nitrogen and in the presence of traces of *tert*-butylcatechol as polymerization inhibitor, using tightly sealed screw-capped vials. Benzene was dried over CaH_2 and distilled prior to use. Pig liver esterase [PLE, 3.1.1.1; suspension in 3.2 M $(NH_4)_2SO_4$ solution, pH 8; 185 units/mg of protein] for the enantioselective enzyme-catalysed hydrolysis of compound **5g** was purchased from Sigma Chemical Co., St Louis, USA. The enzymic reactions were performed with a Mettler DL21 pH-stat automatic titrator.

Synthesis of 4′-benzyl-2′,3′-dideoxy-6-*N*-phthaloyl-4′-aza-adenosine 5e

Paraformaldehyde (154.5 mg, 5.15 mmol) and *N*-benzylhydroxylamine **1c** (500 mg, 1.13 mmol) were added to a solution of 6-*N*-phthaloyl-9-vinyladenine **4b** (300 mg, 1.03 mmol) in dry benzene (10 cm^3). After 60 h at 110 °C, the reaction mixture was cooled and evaporated to dryness; purification of the recovered crude material by SCFC ($CHCl_3$–MeOH 90:10, v/v) gave *title product* **5e** as a powder (30.8 mg, 7%), mp 206–208 °C; δ_H 2.96–3.68 (m, 4 H, 2′- and 3′-H_2), 4.11 (s, 2 H, CH_2Ph), 6.58 [dd, $^3J_{cis}$(H1′,H2′) 7.2, $^3J_{trans}$(H1′,H2′) 4.9, 1 H, 1′-H], 7.28–7.34 (m, 5 H, ArH), 7.83 (m, 2 H, H^a Phthaloyl), 8.02 (m, 2 H, H^b Phthaloyl), 8.47 (s, 1 H, 2-H) and 9.02 (s, 1 H, 8-H); FAB$^+$MS, *m*/*z* (%) 427 (45) [MH$^+$], 266 (100) and 162 (37) (Found: C, 64.7; H, 4.2; N, 19.6. $C_{23}H_{18}N_6O$ requires C, 64.8; H, 4.3; N, 19.7%).

Synthesis of 4′-benzyl-2′,3′-dideoxy-6-*N*-dimethoxytrityl-4′-azaadenosine 5f

Paraformaldehyde (35 mg, 1.3 mmol) and *N*-benzylhydroxylamine **1c** (80 mg, 0.29 mmol) were added to a solution of 6-*N*-dimethoxytrityl-9-vinyladenine **4c** (120 mg, 0.26 mmol) in dry benzene (7 cm^3). After 72 h at 108 °C, the reaction mixture was cooled and evaporated to dryness; purification of the recovered crude material by SCFC ($CHCl_3$–MeOH 90:10, v/v) gave title product **5f** as a glassy solid (120 mg, 77%), δ_H 2.67–3.65 (m, 4 H, 2′- and 3′-H_2), 3.79 (s, 6 H, OCH_3), 4.12 (s, 2 H, CH_2Ph), 6.35 [dd, $^3J_{cis}$(H1′,H2′) 8.1, $^3J_{trans}$ (H1′,H2′) 5.2, 1 H, 1′-H], 6.92 (s_{broad}, 1 H, 6-NH), 7.21–7.35 (m, 10 H, ArH), 7.65–7.78 (m, 8 H, ArH), 8.21 (s, 1 H, 2-H) and 8.58 (s, 1 H, 8-H); FAB$^+$MS, *m*/*z* (%) 599 (43) [MH$^+$], 438 (58) and 303 (100).

Synthesis of 4′-benzyl-2′,3′-dideoxy-4′-azaadenosine 5c

Compound **5f** (50 mg, 0.084 mmol) was treated with a 5% chloroform solution of trifluoroacetic acid (TFA, 1 cm^3) at room temp. for 15 min. Saturated aq. $NaHCO_3$ (3 cm^3) was added and the reaction mixture was extracted with chloroform (3 × 2 cm^3). The organic phase was dried over Na_2SO_4 and the

solvent was distilled off under reduced pressure. The recovered solid crude material was washed with *n*-hexane several times in order to eliminate the dimethoxytrityl alcohol formed during the deprotection step. Pure *title product* **5c** was obtained as a solid (21 mg, 84%), mp 175–178 °C; δ_H 2.65–3.66 (m, 4 H, 2′- and 3′-H_2), 4.07 (s, 2 H, CH_2Ph), 6.42 [dd, $^3J_{cis}$(H1′,H2′) 7.5, $^3J_{trans}$(H1′,H2′) 5.2, 1 H, 1′-H], 6.97 (s_{broad}, 2 H, 6-NH_2), 7.27–7.31 (m, 5 H, ArH), 8.15 (s, 1 H, 2-H) and 8.25 (s, 1 H, 8-H); FAB$^+$MS, *m*/*z* (%) 297 (70) [MH$^+$], 136 (100) and 91 (57) (Found: C, 60.7; H, 5.3; N, 28.2. $C_{15}H_{16}N_6O$ requires C, 60.8; H, 5.4; N, 28.4%).

Synthesis of 4′-benzyl-3′-butoxycarbonyl-2′,3′-dideoxy-4′-aza-adenosine 5g

A solution of 9-vinyladenine **4a** (300 mg, 1.86 mmol) and *N*-benzyl-*C*-(butoxycarbonyl)nitrone **7** (481 mg, 2.05 mmol) in dry benzene (10 cm^3) was kept at 104 °C for 96 h. The reaction mixture was evaporated to dryness and purification of the recovered crude material by recrystallization from hot methanol gave the *title product* **5g** as a light yellow solid (332.3 mg, 45%), mp 180–182 °C; δ_H 0.91 (t, 3 H, CH_3), 1.37 (m, 2 H, CH_2CH$_3$), 1.60 (m, 2 H, CH_2CH$_2$CH$_3$), 3.08 [ddd, ^2J(H2′,H2′) 14.0, $^3J_{cis}$(H1′,H2′) 7.8, $^3J_{cis}$(H2′,H3′) 7.7, 1 H, 2′-*H*H], 3.42 [ddd, ^2J(H2′,H2′) 14.0, $^3J_{trans}$(H1′,H2′) 4.5, $^3J_{trans}$(H2′,H3′) 5.8, 1 H, 2′-H*H*], 4.12 (t, 2 H, OCH_2), 4.16 (d, 2 H, CH_2Ph), 4.41 [dd, $^3J_{cis}$(H2′,H3′) 7.7, $^3J_{trans}$(H2′,H3′) 5.8, 1 H, 3′-H], 6.40 [dd, $^3J_{cis}$(H1′,H2′) 7.8, $^3J_{trans}$(H1′,H2′) 4.5, 1 H, 1′-H], 7.27–7.31 (m, 5 H, ArH), 7.37 (s_{broad}, 2 H, 6-NH_2), 8.18 (s, 1 H, 2-H) and 8.38 (s, 1 H, 8-H); FAB$^+$MS, *m*/*z* (%) 397 (25) [MH$^+$], 262 (8), 233 (5), 226 (3), 220 (7), 164 (21), 162 (18), 91 (100) and 77 (34) (Found: C, 60.5; H, 6.0; N, 21.1. $C_{20}H_{24}N_6O_3$ requires C, 60.6; H, 6.1; N, 21.2%).

PLE-catalysed hydrolysis of the racemic ester 5g. Synthesis of (−)-4′-benzyl-3′-carboxy-2′,3′-dideoxy-4′-azaadenosine 5h

The following procedure is representative. A solution of compound **5g** (100 mg, 0.25 mmol) in CH_3CN (2.5 cm^3) was added to a rapidly stirred suspension of PLE (0.27 cm^3) in 0.5 M KH_2PO_4 buffer (7 cm^3) at pH 8 and 26 °C. The pH of the reaction mixture was maintained at 8 by pH-stat-controlled addition of 0.1 M aq. NaOH. The reaction was allowed to proceed until the half-conversion point (418 min), as determined by the volume of base added (1.27 cm^3), had been achieved. Chloroform (10 cm^3) was added and evaporation of the dried (Na_2SO_4) organic layer yielded the unchanged ester. The pH of the remaining mixture was then adjusted to 3.1 by addition of 0.1 M HCl. The aqueous phase was extracted with chloroform (5 × 10 cm^3), and evaporation of the dried (Na_2SO_4) organic solution to dryness gave pure *title acid* **5h** as a solid (72.5 mg, 85%), mp 121–123 °C; $[\alpha]_D$ −43.3 (*c* 1, DMSO); δ_H 2.91 [ddd, ^2J(H2′,H2′) 14.0, $^3J_{trans}$(H1′,H2′) 2.6, $^3J_{trans}$(H2′,H3′) 7.2, 1 H, 2′-H_2], 3.25–3.35 (m, 1 H, 2′-H_2), 3.76 [dd, $^3J_{trans}$(H2′,H3′) 7.2, $^3J_{cis}$(H2′,H3′) 9.6, 1 H, 3′-H], 3.98 (d, 1 H, C*H*HPh), 4.28 (d, 1 H, CH*H*Ph), 6.49 [dd, $^3J_{cis}$(H1′,H2′) 8.1, $^3J_{trans}$(H1′,H2′) 2.6, 1 H, 1′-H], 7.23–7.31 (m, 5 H, ArH), 7.40 (s_{broad}, 2 H, 6-NH_2), 8.15 (s, 1 H, 2-H), 8.37 (s, 1 H, 8-H) and 11.3 (s, 1 H, CO_2H); FAB$^+$MS, *m*/*z* (%) 341 (12) [MH$^+$], 279 (7), 261 (5), 205 (8), 164 (15), 162 (27), 91 (72) and 77 (100) (Found: C, 56.4; H, 4.7; N, 24.6. $C_{16}H_{16}N_6O_3$ requires C, 56.5; H, 4.7; N, 24.7%).

Acknowledgements

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