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9-Vinylguanine: an easy access to aza-analogs of 2′,3′-dideoxyguanosine

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Abstract—9-Vinylguanine, obtained for the first time and fully characterised by X-ray analysis, allows access to aza-analogues of 2′,3′-dideoxynucleosides through cycloaddition processes. © 2001 Elsevier Science Ltd. All rights reserved.

In the last years there has been much effort to develop efficient treatment against HIV infection. Good results were obtained by using a combination of agents that inhibit different steps in the HIV life cycle (reverse transcriptase and protease inhibitors). In several clinical trials (CPCRA, DELTA, etc.) this strategy showed a considerable delay in progression of the disease and prolonged survival in patients with advanced HIV.[1]

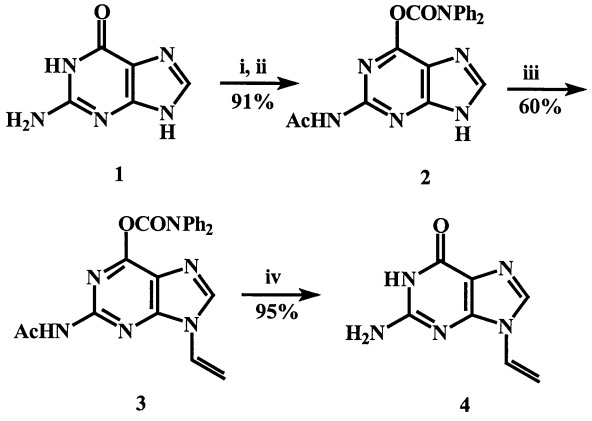
Modified nucleosides (AZT, ddC, etc…) have, very often, shown good antiviral properties pushing the interest towards other related systems like, for example, isoxazolidinyl nucleosides.[2] Synthesis of 4′-aza analogues of 2′,3′-dideoxynucleosides was proposed as a potential way to reach this aim.[3,4] We have already reported a valuable strategy for the preparation of isoxazolidine nucleosides of thymine, adenine and cytosine, and we found that the 4′-aza-2′,3′-dideoxy-erythrofuranosyl derivative of thymine acts against HIV 'in vitro' test.[3] Considering the antiviral activities showed by other guanine analogues in similar homologous series [i.e. acyclovir, DMPG, Carbovir, etc.],[5–7] the synthesis of deoxyguanosine derivatives of isoxazolidinyl nucleosides seems quite interesting.

The key intermediate to take advantage of using the method we previously described[3] is certainly 9-vinylguanine, already isolated and characterised as a radiation-induced degradation product of 2′-deoxyguanosine,[8] but never synthesised before. In fact the methods proposed, so far, consist of relatively low yielding, multistep procedures, which only give a precursor of the desired substrate.[9–11] Moreover attempts to use the direct alkylation of guanine with ethylene oxide resulted in the 7-alkyl derivative.[10,12]

On the other hand the direct *N*-vinylation of guanine gives approximately equal amounts of the *N*-7 and *N*-9-vinyl products.[11,12]

Since 2-*N*-acetyl-6-*O*-diphenylcarbamoylguanine was successful in giving highly regioselective coupling with acetylated pentafuranoses and α-halo ethers,[13] we decided to use the same substrate in vinyl exchange between vinyl acetate and a nitrogen heterocyclic compound using vinyl acetate in a valuable one-step preparation of *N*-vinylheterocycles (Scheme 1).

The reaction sequence gives product **4** without detecting any *N*-7 contaminant as shown by the absence of any other signals in the ^{1}H NMR spectrum. These data are in accordance with the previous observations that the signals of H_8 and NH_2 for the *N*-9 isomer are respectively shifted upfield



i: $(CH_3CO)_2O$, py; ii: Ph_2NCOCl, py; iii: CH_2=CHOAc, H^+, $(AcO)_2Hg$; iv: NH_3, MeOH.

Scheme 1.

Keywords: vinylation; purines; cycloadditions; antivirals.

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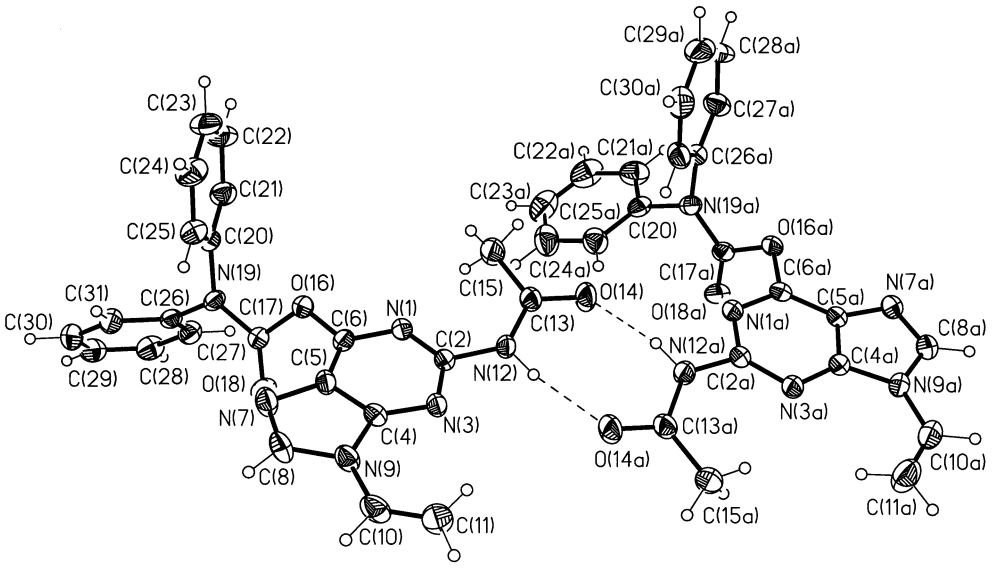
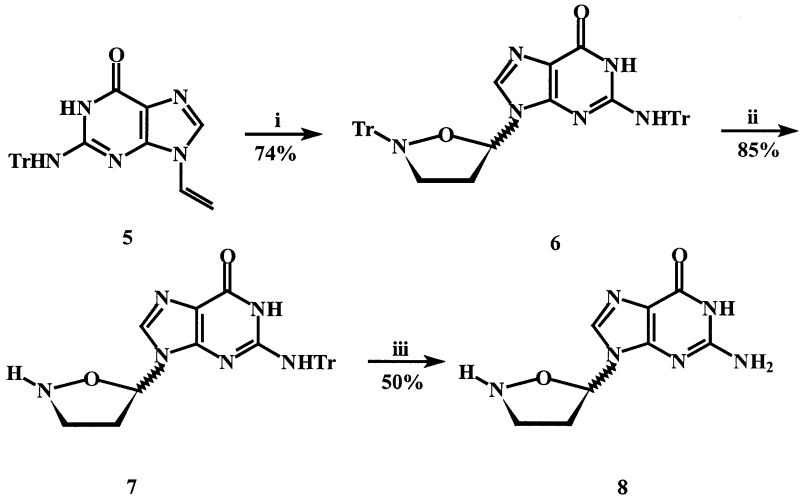


Figure 1.



i: Ph_3CNHOH, CH_2O, toluene; ii: CF_3COOH 5% in CH_2Cl_2, CF_3CH_2OH; iii: CF_3COOH 15% in CH_2Cl_2, CF_3CH_2OH

Scheme 2.

and downfield relative to the signals of H_8 and NH_2 signals or the *N*-7 isomer.[8,14]

The structure of **3** was proven by X-ray analysis (Fig. 1).[15]

Finally the tritylated isomer **5** was devised in order to exploit a cycloaddition process leading to a fully acid-labile protected azanucleoside and afforded **8** after complete deblocking with trifluoroacetic acid, according to Scheme 2.

The presented method allows the easy synthesis of 9-vinylguanine which is now available for the preparation of nucleoside derivatives obtained using their vinyl synthons.

1. Experimental

1.1. General

Silica gel-pre-coated plates were used for TLC and Kieselgel 60 H without gypsum was used for short-column chromatography. HPLC system: HP 1100 (Hewlett–Packard), sample loop: 2.0 ml, column 250×4.6 mm Jupiter C_{18} 10 μ Phenomenex; detection: UV 230 nm, Hewlett–Packard (Variable Wavelength). Solvent (A): 0.1% TFA aq.; solvent (B) MeOH; gradient: A 100% for 5 min, 10% B in A in 2 min., 30% B in A 5 min.; and after 5 min, 100% A in 5 min.; flow 3.0 ml min^{-1}. IR spectra were determined as film on KBr on PE Paragon 1000 PC FT-IR Spectrometer.

Melting points were obtained on a Kofler apparatus. NMR spectra were obtained on WM-300 Bruker spectrometer with tetramethylsilane as internal standard and DMSO-d_6 or $CDCl_3$ as solvents, J values are given in Hz. Structural assignments have been made after extensive decoupling experiments. FAB-Mass spectra were obtained on a VG-micromass ZAB 2F mass spectrometer, from a 2 mm^3 *m*-nitrobenzyl alcohol or glycerol solution of sample by using the standard gun operated with neutral Xenon beam of 8 keV and a neutral current of 10 μA.

1.1.1. 2-(*N*-Acetyl)-6-(*O*-diphenylcarbamoyl) guanine (2). Guanine (10.0 g; 66.2 mmol) and dry *N*,*N*-dimethylacetamide (85 mL) were placed in a 250 mL flask fitted with a reflux condenser under dry nitrogen. Acetic anhydride (20 mL; 211.6 mmol) was added to this suspension. The solution was warmed to 160°C. When the reaction was completed, it was cooled and the white solid obtained (2,9-diacetylguanine) was filtered and washed with EtOH.

To a suspension of 2,9-diacetylguanine (10 g; 42.5 mmol)), DIPEA (15 mL) and dry pyridine (200 mL), diphenylcarbamoyl chloride (10.88 g, 46.9 mmol) was added. The reaction was stirred for 4 h (TLC: CH_2Cl_2/MeOH 9:1 v/v). Then H_2O (20 mL) was added and the mixture was stirred for 10 min. The solvents were removed in vacuo. The residue was placed in a solution of H_2O and EtOH (1:1, v/v) and heated to 70°C. After 3.5 h the suspension was cooled and filtered. A white solid was obtained (23.36 g, 91%); mp 153–155°C; ν_{max} (KBr) 3387, 1769, 1596, 1470; δ_H (DMSO-d_6): 2.18 (s, 3H; CH_3CO), 7.26–7.56 (m, 10H; $N(C_6H_5)_2$), 8.46 (s, 1H; 8-CH); FAB-MS (+), NBA *m/z*: 411 $[M+Na]^+$ (23%), 389 $[M+H]^+$ (29%), 196 $[CONPh_2]^+$ (100%); anal. calcd. for $C_{20}H_{16}N_6O_3$ C 61.85, H 4.15, N 21.64 found C 61.79, H 4.20, N 21.69.

1.1.2. 2-(*N*-Acetyl)-6-(*O*-diphenylcarbamoyl)-9-vinylguanine (3). A solution of sulfuric acid (0.25 mL) in AcOEt (12 mL) was added to a suspension of $Hg(OAc)_2$ (0.49 g; 1.5 mmol) in vinyl acetate (48 mL; 1.5 mmol). After two minutes, a clear solution appeared. Then, 2-(*N*-acetyl)-6-(*O*-diphenylcarbamoyl) guanine (6.0 g; 15.0 mmol) in DMF (80 mL) was added. As a polymerisation inhibitor hydroquinone (0.1 g) was added. The mixture was refluxed for 6 h to allow the reaction to be finished. Then aqueous $NaHCO_3$ (5% w/w) was added up to neutralisation. A white solid was formed, filtered; the brown filtrate extracted with Et_2O (3×50 mL), the organic layer dried over sodium sulphate and concentrated in vacuo. The crude product was purified by flash chromatography with $CHCl_3$/MeOH (97.5:2.5 v/v). A yellow solid was obtained (3.72 g, 60%); mp 187–188°C; ν_{max} (KBr) 3210, 1734, 1001, 906; δ_H ($CDCl_3$): 2.53 (s, 3H; CH_3), 5.19 (dd, 1H; 2′-CH_{cis}, J_{cis}=9.3, J_{gem}=1.8), 5.91 (dd, 1H; 2′-CH_{trans}, J_{trans}=16.0, J_{gem}=1.8), 7.10 (dd, 1H; 1′-CH, J_{trans}=16.0, J_{cis}=9.3), 7.20–7.55 [m, 10H; $N(C_6H_5)_2$],), 8.12 (s, 1H; 8-CH), 8.25 (brs, 1H; 2-NH); FAB-MS (+), NBA *m/z*: 437 $[M+Na]^+$ (4%), 415 $[M+H]^+$ (15%), 196 $[CONPh_2]^+$ (100%); anal. calcd. for $C_{22}H_{18}N_6O_3$ C 63.76, H 4.38, N 20.28 found C 63.83, H 4.36, N 20.27.

1.1.3. 9-Vinylguanine (4). In a solution of **3** (2.5 g; 6.0 mmol) and MeOH (60 mL) ammonia was bubbled for 10 min and then the suspension was warmed to 60°C for 1 h. When the reaction was completed (TLC: $CHCl_3$/MeOH 9:1 v/v), the solvent was removed under reduced pressure. The residue was suspended in CH_2Cl_2 and stirred for 20 min. Then it was filtered and washed with CH_2Cl_2. A white solid was obtained (1.01 g, 95%); mp>300°C; ν_{max} (KBr) 3317, 3165, 1698, 998, 892; δ_H (DMSO-d_6): 5.05 (d, 1H; 2′-CH_{cis}, J_{cis}=9.39), 5.87 (d, 1H; 2′-CH_{trans}, J_{trans}=16.12), 6.70 (brs, 2H; 2-NH_2), 7.08 (dd, 1H; 1′-CH, J_{trans}=16.12, J_{cis}=9.39), 8.12 (s, 1H; 8-CH), 10.74 (brs, 1H; 1-NH); FAB-MS (+), Gly *m/z*: 270 $[M+gly+H]^+$ (9%), 178 $[M+H]^+$ (100%), 152 (33%); anal. calcd. for $C_7H_7N_5O$ C 47.46, H 3.98, N 39.53 found C 47.40, H 4.02, N 39.57.

1.1.4. 2-(*N*-Trityl)-9-vinylguanine (5). 4 (1.5 g, 8.4 mmol) was suspended in dry pyridine (100 mL). Trityl chloride (3.7 g, 13.0 mmol) was added to this suspension. The reaction solution was warmed to 90°C until TLC ($CHCl_3$/MeOH 90:10 v/v) showed no starting material. After stirring for 5 h the solution was allowed to cool, 6 mL of water was added and the mixture was stirred for 20 min. The solvents were removed under reduced pressure and the crude product was coevaporated with toluene and then with ethanol, providing a solid residue. Aqueous $NaHCO_3$ (5% w/w, 25 mL) was added and aqueous layer was extracted with $CHCl_3$ (3×20 mL). The organic layer was dried by sodium sulphate and concentrated in vacuo. The crude product was purified by flash chromatography. A white solid was obtained with yield of 75% (2.63 g); mp 278–280°C; ν_{max} (KBr) 3220, 1706, 989, 900; δ_H (DMSO- d_6): 4.65 (d, 1H, 2′-CH_{cis}, J_{cis}=9.21); 5.28 (d, 1H, 2′-CH_{trans}, J_{trans}=16.11); 6.56 (dd, 1H, 1′-CH, J_{trans}=16.11, J_{cis}=9.21); 7.20–7.34 (m, 15H, ArH); 7.78 (s, 1H, 2-NH); 7.89 (1H, s, 8-CH); 10.71 (1H, brs, 1-NH); FAB-MS (+), m-NBA, *m/z*: 442 $[M+Na]^+$ (61%), 420 $[M+H]^+$ (100%), 342 $[M+H-C_6H_6]^+$ (8%); anal. calcd. for $C_{26}H_{21}N_5O$ C 74.44, H 5.05, N 16.70 found C 74.45, H 5.08, N 16.68.

1.1.5. 4′-Aza-4′-(*N*-trityl)-2′,3′-dideoxy-2-(*N*-trityl) guanosine (6). 5 (1.0 g, 2.3 mmol) was added under dry nitrogen to a suspension of N-tritylhydroxylamine (1.6 g, 5.7 mmol) and paraformaldehyde (0.21 g, 6.90 mmol) in dry toluene (60 mL). Some crystals of hydroquinone were added as a polymerisation inhibitor. The mixture was stirred and heated under reflux for 5 h (TLC: CH_2Cl_2/MeOH 95: 5 v/v). Then the solvent was removed under reduced pressure and the residue was coevaporated with ethanol to remove traces of toluene. The crude product was purified by short column chromatography. Yield: 74% (1.20 g); mp 189–190°C; ν_{max} (KBr) 3322, 3036, 1684, 742, 702; δ_H ($CDCl_3$): 1.25–1.30 (m, 1H, 3′-CH); 1.42–1.62 (m, 1H, 2′-CH); 2.74–2.95 (m, 1H, 2′-CH); 3.10–3.28 (m, 1H, 3′-CH); 5.11–5.31 (m, 1H, 1′-CH); 6.90–7.68 (m, 31H, ArH + 2-NH); 7.95 (s, 1H, 8-CH); 11.72 (brs, 1H, 1-NH); FAB-MS (+), m-NBA *m/z*: 729 $[M+Na]^+$ (48%), 707 $[M+H]^+$ (100%), 486 $[M-Tr+Na]^+$ (36%), 464 $[M-Tr+H]^+$ (12%); anal. calcd. for $C_{46}H_{38}N_6O_2$ C 78.16, H 5.42, N 11.89 found C 78.25, H 5.39, N 11.86.

1.1.6. 4′-Aza-2′,3′-dideoxy-2-(*N*-trityl) guanosine (7). To **6** (0.50 g, 0.7 mmol) trifluoroacetic acid (10 mL, 5% dichloromethane solution) and trifluoroethanol (1 mL) were added at room temperature for 5 min. The reaction

was stirred until TLC ($CHCl_3$/MeOH 8:2 v/v) showed no starting material. Aqueous $NaHCO_3$ (5% w/w, 5 mL) was added to solution that was extracted with $CHCl_3$ (3×15 mL). The organic layer was dried over sodium sulfate and the solvents were removed under reduced pressure. The residue was purified by flash chromatography. Yield: 85% (0.28 g); mp 230–232°C; ν_{max} (KBr) 3320, 3202, 1702, 740, 700; δ_H (DMSO-d_6): 1.91–2.52 (m, 4H, 3′-CH + 2′-CH); 5.68–5.77 (brs, 1H, 4′-NH); 5.75–5.98 (m, 1H, 1′-CH); 6.95–7.50 (m, 15H, ArH); 7.70 (s, 1H, 8-CH); 7.80 (s, 1H, 2-NH); 10.73 (brs, 1H, 1-NH); FAB-MS (+), m-NBA, *m/z*: 487 $[M+Na]^+$ (29%), 464 $[M+H]^+$ (73%), 394 (30%), 316 (10%), 243 (100%), 165 (63%), 152 (43%); anal. calcd. for $C_{27}H_{24}N_6O_2$ C 69.81, H 5.21, N 18.09 found C 69.89, H 5.22, N 18.04.

1.1.7. 4′-Aza-2′,3′-dideoxyguanosine (8). 7 (0.50 g; 1.0 mmol) was treated with a 15% dichloromethane solution of trifluoroacetic acid (10 mL) and trifluoroethanol (1 mL) at room temperature for 10 min. When the reaction was finished ($CHCl_3$/MeOH 8:2 v/v), some drops of Et_3N were added to the solution and evaporated to dryness under reduced pressure. The crude product was purified by preparative HPLC (gradient MeOH/H_2O). A white solid was obtained (0.11 g, 50%); mp 287–289°C; ν_{max} (KBr) 3302, 3155, 1683; δ_H (DMSO-d_6): 2.64–2.82 (m, 1H, 2′-CH); 2.83–2.94 (m, 1H, 2′-CH); 3.35–3.47 (m, 1H, 3′-CH); 3.61–3.80 (m, 1H, 3′-CH); 6.18–6.60 (brs, 1H, 4′-NH); 6.47 (dd, 1H, 1′-CH, J_{trans}=7.3, J_{cis}=3.6); 6.81 (brs, 1H, 2-NH_2); 8.13 (s, 1H, 8-CH); 11.08 (s, 1H, 1-NH). FAB-MS (+), m-NBA, *m/z*: 245 $[M+Na]^+$ (20%), 223 $[M+H]^+$ (6%), 152 (18%), 72 (100%). Anal. calcd. for $C_8H_{10}N_6O_2$ C 43.24, H 4.54, N 37.82 found C 43.22, H 4.51, N 37.85.

1.2. X-Ray crystal structure analysis

Brucker R3m/V automatic diffractometer, Mo-K_α radiation, λ=0.71073 Å, graphite monochromator, 295 K, Lorentz-polarization corrections. Data collection, solution and refinement: $\omega-2\theta$, standard methods and subsequent Fourier recycling, SHELXTL-PLUS computer program. $C_{22}H_{18}N_6O_3$, triclinic, space group *P*, *a*=8.635 (2), *b*=14.629 (3), *c*=17.779 (2), α=66.77 (2), β=83.73 (2), γ=82.5 (2)°, *U*=2042.0 (8) $Å^3$, *Z*=4, D_c=1.348 g cm^{-3}, crystal size 0.52×0.48×0.45 mm. 7968 reflections measured in the range $3<2\theta<50°$, 7242 unique and 5344 assumed as observed with $I>3\sigma(I)$; *R*=0.053 and R_w=0.059. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC) as supplementary publication number CCDC 153438. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44-(0)1223-336033 or e-mail: deposit@CCDC.cam.ac.uk].

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15. Two independent molecules [N(1) and N(1a)] constitute the asymmetric unit in the crystallographic cell of compound **3**, which is more suitable for X-ray analysis than **4**. These molecules are joined together by means of hydrogen bonds in which the N(12) and O(14a), O(14) and N(12a) atoms are involved in such a way as to constitute a supramolecular entity. These entities are held in the crystal by Van der Waals forces. Both first and second molecules show similar C–C and C–N bond distances and differ solely for the fact that C(15) is near to N(1) atom in the first whereas the corresponding C(15a) is near N(3a) in the second one.The value of the dihedral angles formed by C(4)–C(5) N(7)–C(8)–N(9) and N(9)–C(10)–C(11), C(4a)–C(5a) N(7a)–C(8a)–N(9a) and N(9a)–C(10a)–C(11a) of 4.9 and 4.2° respectively, ensures that the vinyl moiety is nearly coplanar with the imidazole portion of the purine ring. The establishment of an extended π system lowers the LUMO of the exocyclic double bond thus favouring the cycloaddition approach.