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# A Simple Preparation of N-Vinyl Derivatives of DNA Nucleobases.

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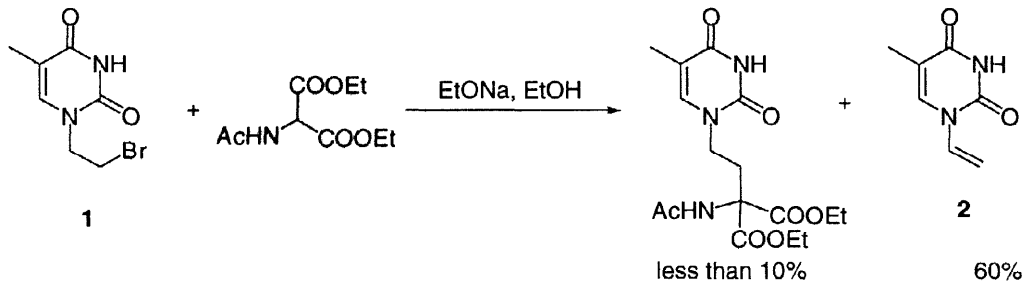
*Abstract*: 1-Vinylpyrimidines and 9-vinylpurines have been prepared via selective alkylation of the heterocyclic ring with 1,2-dibromoethane (or 1,2-dibromopropanol) followed by dehydrobromination with sodium ethoxide in ethanol/ DMF. 

During our work directed towards the synthesis of amino acids carrying nucleobases in the side chain,[1] we were trying to alkylate 1-(2-bromoethyl)-thymine **1** with sodium diethyl acetamidomalonate and we found, as it was probably expected, that the main reaction product was 1-vinylthymine **2**.




Scheme 1

1-Vinylpyrimidines and 9-vinylpurines are important starting materials for the preparation of oligonucleotides[2] and polymeric analogues of nucleic acids and nucleosides,[3] and their synthesis consists in a relatively low yielding multistep procedure.[3,4] For this reason we decided to optimise our procedure and tried to extend its application to all the four DNA nucleobases. We report here a simple procedure (one or two step sequence) to prepare 1-vinylthymine **2**, 9-vinyladenine **4**, 9-vinyl-6-chloro-2-aminopurine **7** (a suitable precursor of the guanine derivative) and 1-vinyl-$N^4$-Cbz-cytosine **11**.

Thymine was selectively alkylated with 4 eq. of 1,2-dibromoethane in DMF in the presence of $K_2CO_3$ to give exclusively 1-(2-bromoethyl)-thymine **1**.[5] This product was isolated in 65% yield and subsequently transformed into 1-vinylthymine **2** through HBr elimination using sodium ethoxide in ethanol/ DMF (82% yield) or $K_2CO_3$ in wet DMF (51% yield). Alternatively **2** was obtained directly from thymine and 1,2-dibromoethane in DMF at 70°C in the presence of 10 equivalents of $K_2CO_3$ (38% yield, see scheme 2). Following this procedure we did not observe the formation of alkylation products at N-3 of thymine (sharp m.p., $^1H$ NMR and $^{13}C$ analysis)[6] or considerable amounts of 1,2-bis(thymidyl)-ethane. [7]

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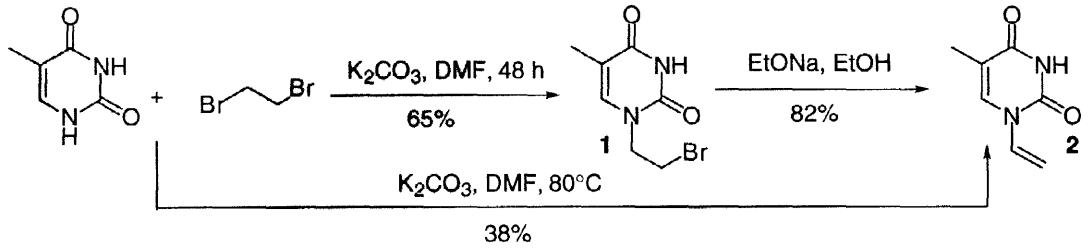
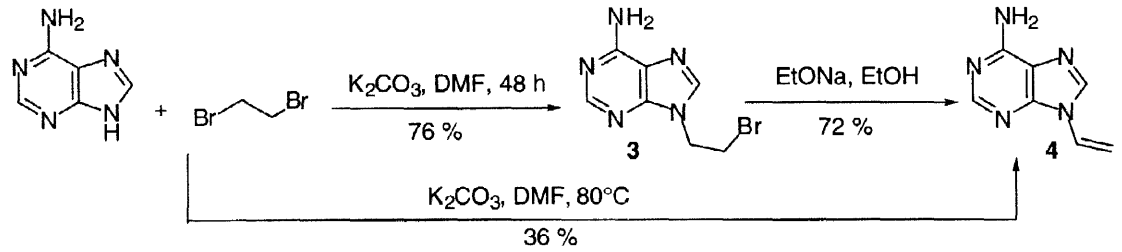


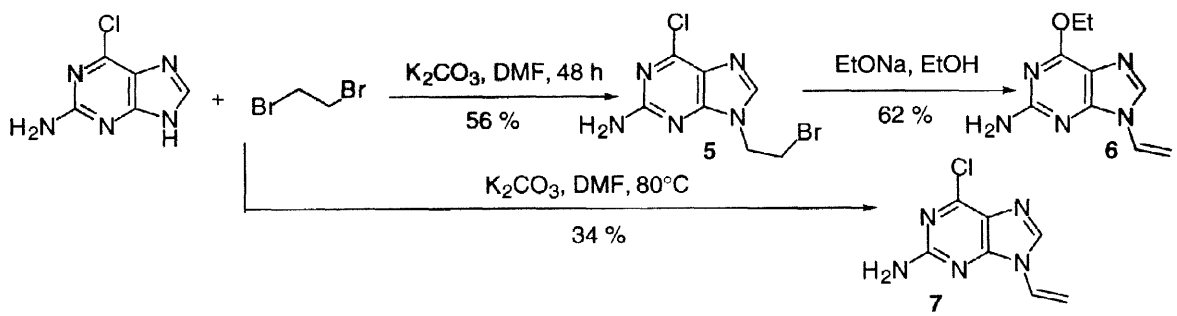

Scheme 2

Adenine reacted analogously to give 9-(2-bromoethyl)-adenine[8] **3**. Compound **4** could be obtained from **3** by dehydrobromination using sodium ethoxide in ethanol (two step procedure but no chromatography, 55% overall yield). Direct transformation of adenine into **4** could be accomplished in 36 % yield after column chromatogaphy using hot DMF and a 10 molar excess of $K_2CO_3$.




Scheme 3

Guanine itself could not be alkylated properly with 1,2-dibromoethane and we were obliged to start from the more expensive 2-amino-6-chloro-purine[9] that was transformed, following the same protocol, into the bromo ethyl derivative **5** in 56 % yield. (Scheme 4) Following our procedure we obtained **5** as a solid compound and other isomers were not detected. The following elimination step with sodium ethoxide worked well although a nucleophilic substitution of the chloropurine occurred giving product **6** in 62 % yield (overall yield 34% and no chromatographic separations).

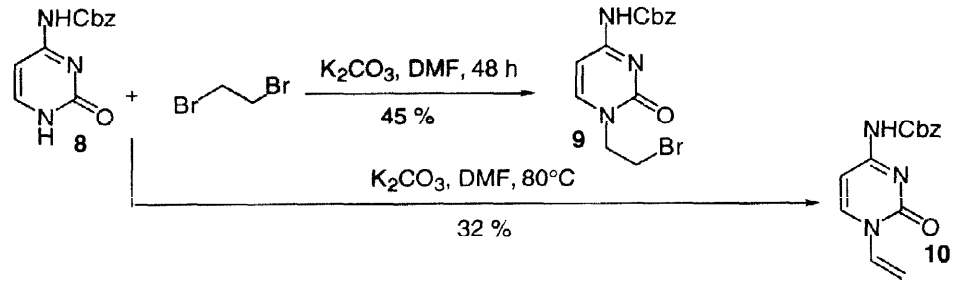



Scheme 4

The 6-chloro derivative **7** was obtained through the one-pot procedure in 34% yield (after column chromatography). Both **6** or **7** could be considered synthetic equivalents of a guanine derivative through an acid hydrolysis of the ethoxy-imine or the chloro-imine group.

Alkylation of free cytosine gave several problems, although small amounts of 1-vinylcytosine could be isolated, after column chromatography, in the reaction of cytosine, 1,2-dibromoethane and $K_2CO_3$ in DMF at 100° for 48 h. Better results were obtained after protection

of the 4-amino position of cytosine as Cbz derivative.[10] Product 8 was easily alkylated at N-1 with 1,2-dibromoethane under standard conditions.

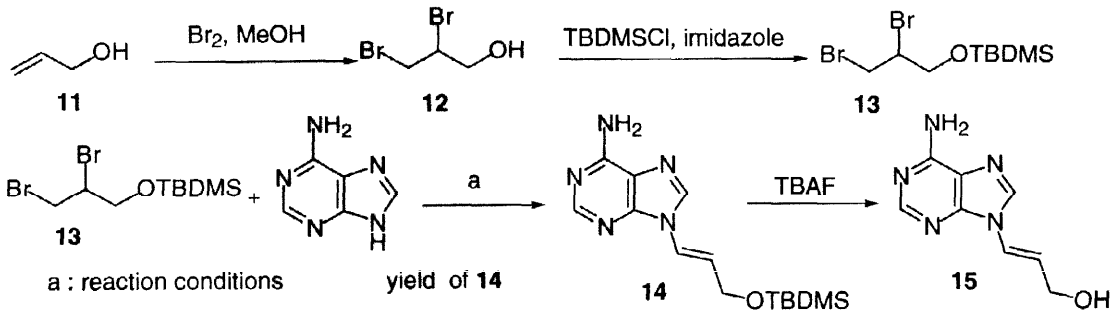



Scheme 5

Product 9 was obtained in 45% yield (after column chromatography). The following dehydrobromination could not be carried out with sodium ethoxide due to partial deprotection at N-4. $N^4$-Cbz-1-vinylcytosine **10** was obtained in 32 % yield in the single step procedure of alkylation-elimination from **8** with $K_2CO_3$ and DMF. (Scheme 5)

We tried also to apply this procedure to the preparation of a substituted vinyl nucleobase. Allylic alcohol **11** was treated with bromine in MeOH to form dibromopropanol **12** that was protected at the oxygen with *t*-BuMe$_2$SiCl (TBDMSCl) in the presence of imidazole. (Scheme 6)

The silylated dibromo derivative **13** was employed for alkylation of adenine. When the reaction was performed in DMF at room temperature, in the presence of $K_2CO_3$, we did not observe the formation of any new product (tlc analysis).




| a : reaction conditions | yield of **14** |
|---|---|
| DMF, $K_2CO_3$, 80°C, 48 h | 23% |
| DMF, $K_2CO_3$, 120°C, 48 h | 12% |
| DMF, $Cs_2CO_3$, 80°C, 48 h | 32% |
| DMF, Py, 80°C, 48 h | --- |
| DMSO, $K_2CO_3$, 80°C, 48 h | 22% |
| DMSO, $Cs_2CO_3$, 80°C, 48 h | 28% |

Scheme 6

When the reaction mixture was heated at 80-90°C, the vinyl-derivative **14** was formed and could be isolated, after column chromatography, in low yield. The analysis of the other fractions of the chromatography did not show the presence of the bromoalkyl intermediate but only some of the starting material **13**. We tried different reaction conditions and found that the product could be obtained exclusively using $K_2CO_3$ or $Cs_2CO_3$ as the base. No reaction was observed using pyridine or other tertiary amines. When DMSO was used, as the solvent, the tlc analysis of the crude mixture showed the complete disappearance of **13** and the formation of **14**. Unfortunately the yield of **14** after chromatographic purification was still low.

Nevertheless using $Cs_2CO_3$ as the base in DMF we could obtain **14** in 32% yield as a single diastereoisomer (E, $^1H$ NMR $J_{trans}$ = 15 Hz). That product was further deprotected at the oxygen with TBAF in MeOH/DMF to give **15.** This compound can be considered as a potentially interesting starting material for the synthesis of different nucleosides and its biological activity is currently under screening.

## EXPERIMENTAL PROCEDURES

**1-(2-Bromoethyl)-thymine, 1.** To a solution of thymine (0.5 g, 3.96 mmol) dissolved in DMF ("purum" grade bottle) (8 mL) 1,2-dibromoethane (2.96 g, 15.9 mmol) was added followed by anhydrous potassium carbonate (1.36 g, 9.9 mmol). The mixture was stirred for 48 h at room temperature. The resulting slurry was filtered through a Celite pad and the cake washed two times with DMF (5 mL). The solvent was evaporated in vacuum to give **1** as a white solid (0.6 g, 55 %) m.p.155°-158°C. An analytical sample could be purified by column chromatography on silica gel using $CHCl_3$ : MeOH 10 : 1 as eluent, m.p. 164°C-165°C.$^1H$ NMR (300 MHz, $CDCl_3$) δ 1.96 (d, 3H, J= 1.2 Hz), 4.27 (t, J= 8.5 Hz, 2H), 4.70 (t, J = 8.5 Hz, 2H), 7.52 (d, J = 1.2 Hz, 1H), 9.9 (bs, 1H). $^{13}C$ NMR (75 MHz, $CDCl_3$ ) δ 12.44, 42.45, 66.17, 116.93, 151.01, 158.43, 161.55. Anal Calcd for $C_7H_9BrN_2O_2$ C, 36.07; H, 3.89; N, 12.02. Found C, 36.18; H, 3.80; N, 12.10.

**1-Vinylthymine, 2.** The crude solid obtained in the previous reaction was dissolved in dry DMF (1 mL) and this solution added to a solution of sodium ethoxide (prepared from 100 mg of Na (4.1 mmol) in 5 mL of dry ethanol) at room temperature. After stirring for 2 h at room temperature the solvent was evaporated under vacuum (having care not to heat the flask) and the pale yellow solid residue treated with cold water. The so formed precipitate was filtered, washed with additional cold water and dried to give 0.267 g of 5 (82% yield); m.p. 200°-202°C (lit.[4a] m.p. 205°-207°C.) $^1H$ NMR (300 MHz, DMSO-$d_6$) δ 1.97 (d, J= 1.1 Hz, 3H), 4.96 (dd, Ja= 9.1 Hz, Jb= 1.3 Hz, 1H), 5.10 (dd, Ja=17.2 Hz, Jb=1.3 Hz, 1H), 7.18 (dd, Ja= 17.2 Hz, Jb=9.1 Hz, 1H), 7.28 (d, J= 1.1 Hz, 1H), 9.1 (bs, 1H). $^{13}C$ NMR (75 MHz, $CDCl_3$ ) δ 16.4, 93.6, 101.7, 133.8, 136.2, 154.4, 161.7.

**1-Vinylthymine, 2 (single step procedure).** Thymine (1 g, 7.9 mmol) was dissolved in DMF (43 mL) and water (1 mL). 1,2-Dibromoethane (6.3 g, 34 mmol) was added followed by anhydrous potassium carbonate (9.4 g, 68 mmol). The flask was covered with an aluminium foil to prevent the exposure to the daylight and the mixture was vigorously stirred at 70°C (oil bath) for 72 h (a mechanical stirrer is strongly recommended). After cooling, the mixture was filtered through a Celite path, the cake washed with DMF (2 x 15 mL) and the solvent evaporated under vacuum. The crude (1.5 g) was purified by flash chromatography (eluent $CHCl_3$ : MeOH 10 : 1) to give pure **1** (0.45 g, 38 % yield).

**9-(2-Bromoethyl)-adenine, 3.** M.p. 195-197°C (lit.[7] 195°-200°C dec.). $^1H$ NMR (300 MHz, DMSO-$d_6$) δ 3.91 (t, J = 6 Hz, 2H), 4.53 (t, J= 6 H, 2H), 7.24 (bs, 2H), 8.11 (s, 1H), 8.14 (s, 1H).

**9-Vinyladenine, 4** M.p. 201°C-203°C (lit.[4a] m.p. 196°-197°C).$^1$H NMR (300 MHz, DMSO-$d_6$) δ 5.15 (dd, $J_a$= 7.6 Hz, $J_b$= 1.5 Hz, 1H), 5.8 (bs, 2H), 5.86 (dd, $J_a$= 16.2 Hz, $J_b$= 1.5 Hz, 1H), 7.22 (dd, $J_a$= 16. 2 Hz, $J_b$= 7.6 Hz, 1H), 8.00 (s, 1H), 8.39 (s, 1H).

**2-Amino-9-(2-bromoethyl)-6-chloropurine, 5** M.p. 182-183°C. $^1$H NMR (300 MHz, DMSO-$d_6$) δ 3.88 (t, J= 6.2 Hz, 2H), 4.45 (t, J= 6.2 Hz, 2H), 6.94 (bs, 2H), 8.41 (s, 1H). $^{13}$C NMR (75 MHz, DMSO-$d_6$) δ 32,7; 56.2; 127.9, 144.0; 146.8; 150.2; 154.9.

**2-Amino-6-ethoxy-9-vinylpurine, 6.** M.p. 216°-218°C $^1$H NMR (300 MHz, DMSO-$d_6$) δ 1.31 (t, J= 7Hz, 3H), 3.91 (q, J =7 Hz, 2H7, 5.18 (d-like, J = 9.2 Hz, 1H), 6.09 (d-like, J = 15 Hz, 1H), 7.02 (bs, 2H), 7.15 (dd, $J_a$= 15 Hz, $J_b$= 8 Hz, 1H), 8.24 (s, 1H), 8.44 (s, 1H). $^{13}$C NMR (75 MHz, DMSO-$d_6$) δ 14.9, 66.7, 100.0; 124.8; 128.9, 144.9; 147.3; 151.9; 155.9.

**2-Amino-9-vinyl-6-chloropurine, 7.** M.p. 241°-242°C $^1$H NMR (300 MHz, DMSO-$d_6$) δ 5.10 (d-like, J = 9.2 Hz, 1H), 6.00 (d-like, J = 15.6 Hz, 1H), 6.99 (bs, 2H), 7.13 (dd, $J_a$= 15.6 Hz, $J_b$= 9.2 Hz, 1H), 8.24 (s, 1H), 8.41 (s, 1H). $^{13}$C NMR (75 MHz, DMSO-$d_6$) δ 101.0; 126.8; 129.9, 144.8; 147.8; 151.2; 154.9.Anal Calcd for $C_7H_6ClNO_5$ C, 42.98; H, 3.09; N, 35.80. Found C, 42.48; H, 3.00; N, 35.70

**N$^4$-(Benzyloxycarbonyl)-1-(2-bromoethyl)-cytosine, 9.** Following the general procedure, product **4** was obtained in 45% yield after column chromatography on silica gel (eluent $CHCl_3$ : MeOH 10 : 1) as a waxy material. $^1$H NMR (300 MHz, DMSO-$d_6$) δ 4.17 (t, J= 6.1 Hz, 2H), 4.77 (t, J= 6.1 Hz, 1H), 5.21 (s-like, 2H), 7.17 (d, J=7 Hz, 1H), 7.3 (s like, 5H + 1H), 7.81 (d, J= 7Hz, 1H). $^{13}$C NMR (75 MHz, DMSO-$d_6$) δ 31.7, 53.4, 69.4, 97.2, 127.3; 127.5; 128.7; 131.6; 140.8, 157.3; 159.2; 164.6.

**N$^4$-(Benzyloxycarbonyl)-1-vinylcytosine, 10** Product **10** was obtained in 32% yield after column chromatography on silica gel (eluent $CHCl_3$ : MeOH 10 : 1). M.p. 146°-148°C. $^1$H NMR (300 MHz, DMSO-$d_6$) δ 5.06 (dd, $J_a$= 8.1 Hz, $J_b$= 1.3 Hz, 1H), 5.10 (dd, $J_a$=19.2 Hz, $J_b$=1.3 Hz, 1H), 5.20 (AB system, J= 11 Hz, 2H), 7.10 (d, J=7 Hz, 1H), 7.3-7.4 (m, 5H + 1H + 1H), 7.57 (d, J= 7Hz, 1H). $^{13}$C NMR (75 MHz, DMSO-$d_6$) δ 101.1, 126.6, 127.5; 129.7; 131.0; 141.8, 158.3; 159.2; 163.6. Anal Calcd. for $C_{14}H_{23}N_3O3$ C, 61.99; H, 4.83; N, 15.49. Found C, 61.71; H, 4.85; N, 15.52.

**1-(*tert*-Butyldimethylsilyl)-2,3-dibromopropane, 13.** To a solution of allylic alcohol (1.7 g, 30 mmol) in MeOH (25 mL) cooled to 0°C, bromine (4.8 g, 30 mmol) was slowly added. The mixture was stirred at room temperature for 10 min, than methanol was evaporated under vacuum and the crude dissolved in DMF (10 mL). To this mixture, imidazole (3.9 g, 57 mmol) was added followed by TBDMSCl (4.15 g, 27.5 mmol). The mixture was stirred at room temperature for 48 h. Water (50 mL) was added and the solution extracted with $Et_2O$ (4 x 25 mL each). The organic layers were collected, dried over anhydrous $Na_2SO_4$ the solvent evaporated and the product purified by vacuum distillation to give 7.5 g of **13** (75% yield). B.p. 45-49°C / 0.2 mmHg $^1$H NMR (300 MHz, $CDCl_3$) δ 0.11 (s, 6H), 0.92 (s, 9H), 3.75-3.86 (m of 6 lines, 2H), 3.92 (A part of an ABX system, 1H), 4.05 (B part of an ABX system, 1H), 4.20 (m, 1H).

**9-(3-*tert*-Butyldimethylsilyl-2-propen-1-yl)-adenine, 14.** Adenine (1.6 g, 12 mmol), cesium carbonate (4.0 g, 12 mmol) and **13** (2.0 g, 6 mmol) were mixed in DMF and stirred at 70°C (oil bath) for 48 h. After cooling to rt, the mixture was diluted with water (100 mL) and the solid filtered and purified by column chromatography on silica gel (eluent $CHCl_3$ / EtOH 10 / 1) to give 0.58 g of **14** (32% yield) m.p. 185-188°C. $^1$H NMR (300 MHz, DMSO-$d_6$) δ 0.14 (s, 6H), 0.96 (s, 9H), 4.43 (dd, $J_a$= 4 Hz, $J_b$ =1.5 Hz, 2H), 6.65 (dt, $J_a$= 15 Hz, Jb= 4Hz, 1H), 7.20 (dt, $J_a$= 15 Hz, $J_b$= 1.5

Hz, 1H), 7.28 (bs, 2H), 8.02 (s, 1H), 8.41 (s, 1H). $^{13}C$ NMR (75 MHz, $CDCl_3$) δ -5.7, 14.9, 21.0, 60.8, 113.7, 123.8, 128.9, 143.8, 147.9, 152.7, 156.6. Anal Calcd. for $C_{14}H_{23}N_5OSi$ C, 55.05; H, 7.59; N, 22.93. Found C, 55.35; H, 7.61; N, 22.99.

**9-(3-Hydroxy-2-propen-1-yl)-adenine, 15.** Product **14** (0.3 g, 1 mmol) was dissolved in DMF (1 mL) and TBAF (0.52 g, 2mmol) in MeOH (2 mL) added. The mixture was stirred at room temperature for 12 h, the solvent evaporated and the residue purified by column chromatography (eluent CHCl3 / MeOH 3/ 1) to give 0.14 g of **15** (73 % yield). m.p. 208-210°C $^1H$ NMR (300 MHz, DMSO-$d_6$) δ 3.5 (bs 1H), 4.5 (m, 2H), 6.55 (dt, $J_a$= 15 Hz, $J_b$= 6Hz, 1H), 7.00 (dt, $J_a$= 15 Hz, $J_b$= 1.5 Hz, 1H), 7.28 (bs, 2H), 7.98 (s, 1H), 8.31 (s, 1H). $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 62.8, 115.7, 121.8, 128.9, 145.8, 148.2, 152.7, 154.6.Anal Calcd. for $C_8H_9N_5O$ C, 50.26; H, 4.74; N, 36.63. Found C, 50.45; H, 4.67; N, 36.79.

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## REFERENCES AND NOTES.

1 a) Lenzi, A.; Reginato, G.; Taddei, M.*Tetrahedron Lett.* **1995,** *36,* 1713. b) Ciapetti, P.; Soccolini, F.; Taddei, M. *Tetrahedron,* **1997,** *53,* 1167. c) Ciapetti, P.; Mann, A.; Schoenfelder, A.; Taddei, M.; Trifilieff, E.; Canet, I.; Canet, J.L. *LIPS,* **1997,** 4, 341.

2 Leggio, A.; Liguori, A.; Procopio, A.; Siciliano, C.; Sindona, G *Tetrahedron Lett.* **1996,** *37,* 1277. Gi, H-J.; Xiang, Y.; Schinazi, R.F.; Zhao, K. *J. Org. Chem.* **1997,** *62,* 88 and references therein.

3 Yashima, E.; Tajima, T.; Miyauchi, N. *Biopolymers.* **1992,** *32,* 811. Yashima, E.; Suehiro, N.; Akashi, M.; Miyauchi, N. *Chem. Lett.* **1990,** 1113.

4 a) Ueda, N.; Kondo, K.; Kono, M.; Takemoto, K.; Imoto, M. *Makromol. Chem.* **1968,** *120,* 13. b) Pitha, J.; Ts'o, P.O.P. *J. Org. Chem.* **1968,** 33, 1341. c) Giglio, G.; Napoli, A.; Leggio, A.; Liguori, A.; Procopio, A.; Siciliano, C.; Sindona, G. *Synth. Commun.* **1996,** *26,* 4211. d) Dulcére, J.P.; Baret, N.; Rodriguez, J. *Synlett* **1995,** 923.

5 Browne, D.T.; Eisinger, J.; Leonard, N.J *J. Am. Chem. Soc.* **1968,** *90,* 7302. Kim, M-S.; Gokel, G.W. *J. Chem. Soc. Chem. Commun.* **1987,** 1686.

6 Shaw, G. in *Comprehensive Heterocyclic Chemistry,* A.R. Katritzky, C.W. Rees eds. Pergamon Press, Oxford, 1984, vol 5, 499.

7 This product (5-10%) was isolated by column chromatography during the single step procedure.

8 Flyerat, A.; Demeunynck, M.; Constant, J-F.; Michon, P.; Lhomme, J. *J. Am. Chem. Soc.* **1993,** *115,* 9952.

9 2-Amino-6-chloro-purine can be easily transformed into guanine in strong acidic medium. See ref. 1b.

10 Thomson, S.A.; Josey, J.A.; Cadilla, R.; Gaul, M.D.; Hassman, C.F.; Luzzio, M.J.; Pipe, A.J.; Reed, K.L.; Ricca, D.J.; Wiethe, R.W.; Noble, S.A. *Tetrahedron* **1995,** *51,* 6179.