Synthesis and ^{15}N NMR Characterization of 4-Vinylbenzyl Substituted Bases of Nucleic Acids

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Received January 21, 2003

The following substances have been synthesized and characterized as monomers or intermediates for syntheses of new polymers: 1-(4-vinylbenzyl)uracil (**1a**), 1-(4-vinylbenzyl)thymine (**1b**), *N*-4-acetyl-1-(4-vinylbenzyl)cytosine (**1c**), 1-(4-vinylbenzyl)cytosine (**1d**), 9-(4-vinylbenzyl)adenine (**2a**), 2-amino-9-(4-vinylbenzyl)-6-chloro-9*H*-purine (**2b**), and 9-(4-vinylbenzyl)-guanine (**2c**). The alkylation reactions with 4-vinylbenzyl chloride were catalyzed with anhydrous sodium iodide. The substitution at position 9 in substances **2a-c** was confirmed by ^{15}N NMR.

J. Heterocyclic Chem., **40**, 671(2003).

Introduction.

Purine and pyrimidine bases represent a significant part of nucleic acids, which substantially contribute to the mechanism of storage and transfer of genetic information. The present era is characterized by an intensive development of molecular biology, which necessitates a detailed study of mechanisms at the level of nucleic acids [1]. The template polymerization plays a key role in synthesis of biopolymers, such as DNA, RNA and proteins [1]. In the case of nucleic acids, the template polymerization is characterized by an extremely precise arrangement of the individual bases in biopolymer in accordance with the daughter template, with respect to both the sequence and the chain length [1]. On the other hand, synthetic polymers are usually much less defined from the standpoint of both the control over molecular mass distribution and the polymerization degree. In earlier work [2,3], some monomers for radical template polymerization were prepared on the basis of esters of methacrylic and acrylic acids corresponding to uracil or adenine derivatives, and these were used for preparation of complementary polymers.

The aim of the present work was to synthesize and characterize monomers derived from purine and pyrimidine bases. For the monomer units we chose pyrimidine bases substituted at 1-position and purine bases substituted at 9-position with 4-vinylbenzyl substituent. These monomers will be used to study the possibilities of the template polymerization itself and, moreover, they can also be applied to preparation of a number of modified polymers with unique properties. Another example of application can consist in a modified packing of a chromatographic column that can be used for separation of nucleosides and related compounds [4,5].

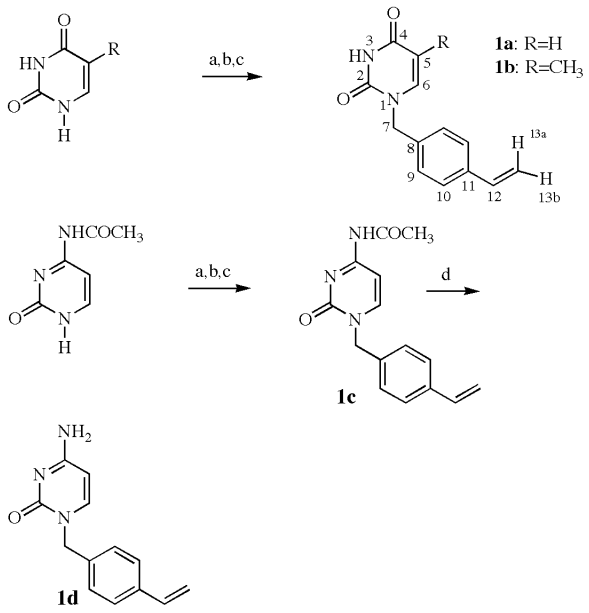
Results and Discussion.

1. Discussion of Synthesis.

The synthesis of 1-(4-vinylbenzyl)uracil (**1a**) and 1-(4-vinylbenzyl)thymine (**1b**) was realized by the method [6] of alkylation of the silylated bases. The silylation of uracil and thymine was achieved by 24-h refluxing with hexamethyldisilazane (HMDS) using trimethylsilyl chloride (TMSC) as the catalyst. However, in our case the alkylation was catalysed with anhydrous NaI with addition of hydroquinone as polymerization inhibitor (Scheme 1). After the alkylation, the silyl groups were removed by hydrolysis: the yields of monomers **1a** and **1b** after crystallization were 55 and 48%, respectively.

1-(4-Vinylbenzyl)cytosine (**1d**) was prepared in analogous way from *N*-4-acetylcytosine: after the silylation, alkylation and hydrolysis we obtained first *N*-4-acetyl-1-(4-vinylbenzyl)cytosine (**1c**), which was subsequently submitted to methanolysis to give **1d** (Scheme 1). The yields of monomers **1c** and **1d** after crystallization were 50 and 77%, respectively.

Scheme 1

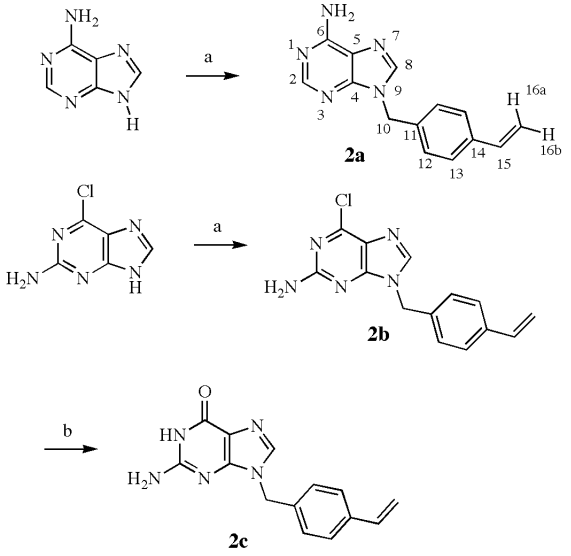


(a) HMDS/TMSC; reflux 24 hrs., (b) 4-vinylbenzylchloride, NaI/DMF 80°C 8-16 hrs., (c) H_2O, r.t./10 min., (d) NH_3/CH_3OH r.t. overnight.

9-(4-Vinylbenzyl)adenine (**2a**) was prepared by analogous method of alkylation of adenine with allyl chloride [7] or benzyl chloride [8]. However, in this case the alkylation of adenine with 4-vinylbenzyl chloride was catalyzed by anhydrous NaI. The synthesis was carried out by boiling in DMF under reflux, in the presence of K_2CO_3 and hydroquinone for 16 hrs (Scheme 2). The minor product – 7-(4-vinylbenzyl)adenine – was not isolated and was separated on silica gel and by crystallization; the overall yield of **2a** was 35%.

9-(4-Vinylbenzyl)guanine (**2c**) was synthesized by alkylation of 2-amino-6-chloro-9*H*-purine in boiling DMF under reflux with added K_2CO_3 and hydroquinone for 32 hrs. 2-Amino-7-(4-vinylbenzyl)-6-chloro-9*H*-purine was not isolated and was separated by flash chromatography on silica gel. The crystallization of evaporated eluate first gave 2-amino-9-(4-vinylbenzyl)-6-chloro-9*H*-purine (**2b**) (33% yield), whereupon the chlorine at 6-position of purine **2b** was substituted (S_N) by hydroxyl ion, which gave 9-(4-vinylbenzyl)guanine (**2c**) in the yield of 64% after recrystallization.

Scheme 2



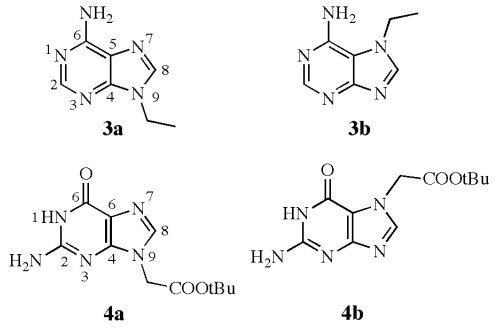
(a) 4-vinylbenzylchloride, K_2CO_3; (NaI only in case of **2a**)/DMF reflux 16-32 hrs, (b) NaOH/r.t. overnight

2. Discussion of NMR Results.

2.1. Purine Derivatives.

As the syntheses of *N*-alkylated purine derivatives usually produce mixtures of N^7 and N^9 regio-isomers, it is important for one to be able to differentiate between these regio-isomers. Application of multinuclear magnetic resonance techniques proved useful for this purpose. Recently, certain empirical rules have been developed, which are based on differences in chemical shifts of certain diagnostic carbon signals with application of 2D ^{1}H-^{13}C NMR spectroscopy [9]. The study of protons and carbon atoms provides an only indirect piece of information about the nitrogen skeleton, which is most sensitive to the substitution type. Therefore, very useful for this purpose appeared to be the techniques based on ^{15}N NMR spectroscopy [10-12]. Thorough ^{15}N NMR studies of selected N^7/N^9-substituted purine analogues carried out by Remaud *et al.*[11] showed substantial differences in electron structures of the N^7/N^9 isomers. This paper [11] and the results published by Marek *et al.*[12] resulted in certain conclusions that are useful in diagnosis of substitution type in purine derivatives. Perhaps the most useful is the chemical shift of N-3 nitrogen. In N^7 this nitrogen atom is less shielded by about 18-20 ppm as compared with the N^9 isomer.

The ^{15}N NMR parameters of synthesized purine derivatives **2a** and **2c** are presented in Table 1. Table 2 presents the ^{15}N NMR parameters of selected N^7 and N^9 isomers for comparison. Figure 1 gives the ^{1}H-^{15}N HMBC spectrum of compound **2c**. A comparison of ^{15}N chemical shifts of compounds **2a** with **3a**, and **2c** with **4a**, shows a good accordance between the individual derivatives. Wherefrom it follows that the substances **2a,c** prepared by us are N^9 substituted purine derivatives.



2.2 Pyrimidine Derivatives.

The nitrogen chemical shifts of compounds **1a-d** are given in Table 1. The ^{1}H-^{15}N *gs* HMBC spectrum of thymine derivative **1b** is presented in Figure 2. On the whole, the results are comparable with the data obtained on analogous nucleosides [13,14].

The crystal structures of 1-(4-vinylbenzyl)uracil (**1a**) and 9-(4-vinylbenzyl)adenine (**2a**) appeared [15] during completion of our work.

EXPERIMENTAL

Measurement of NMR Spectra.

The ^{1}H and ^{13}C NMR spectra were measured on a Bruker AMX 360 apparatus equipped with a 5 mm broadband probe and on a Bruker Avance 500 apparatus equipped with a 5 mm broadband

probe with Z-gradients and a with triple resonance probe with Z-gradients (360.14 resp. 500.13 MHz for ^{1}H and 90.57 resp 125.77 MHz for ^{13}C). For the measurement, the substances were dissolved in hexadeuteriodimethyl sulfoxide (DMSO-d_6). The

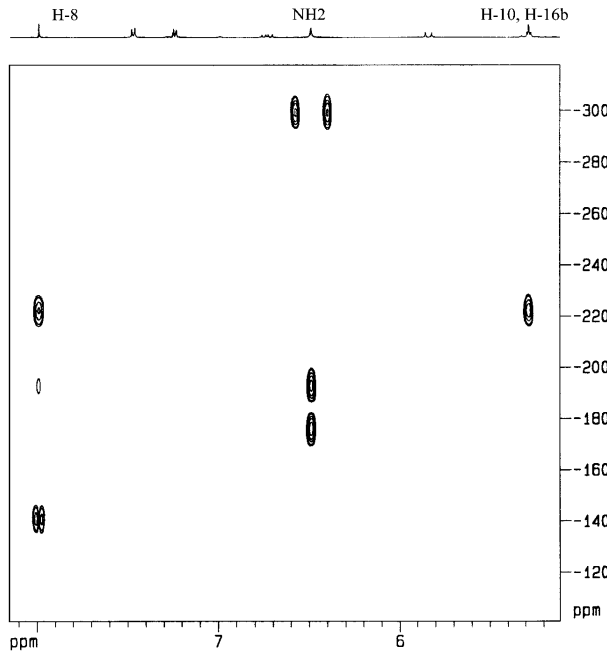


Figure 1. ^{1}H–^{15}N HMBC spectrum of **2c**.

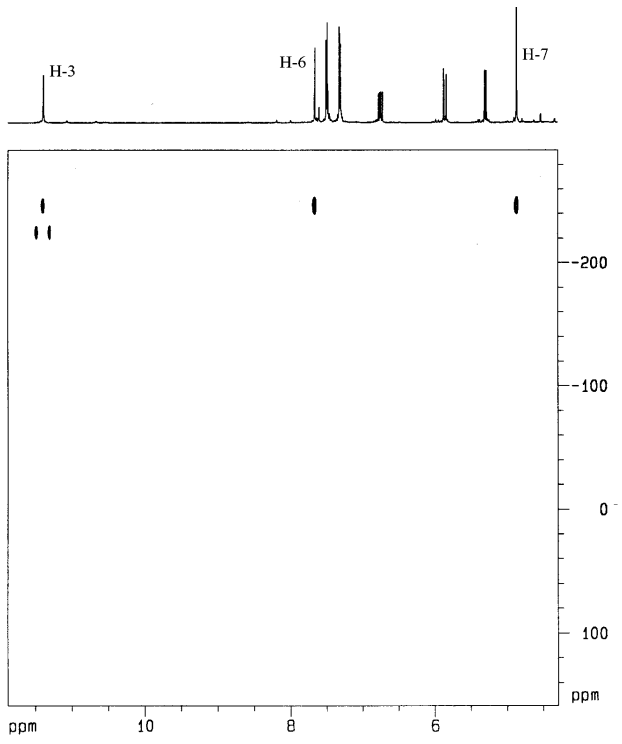


Figure 2. ^{1}H–^{15}N HMBC spectrum of **1b**.

Table 1

^{15}N Chemical Shifts of 4-Vinylbenzyl Substituted Bases of Nucleic Acids

Compound	N-1	N-3	N-7	N-9	NHR
1a	-243.1	–222.1			
1b	-246.6	–224.8			
1c	-218.5	-145.3			–231.5
1d	-233.8	[a]			–288.6
2a	–146.2	–156.6	–140.7	–219.1	[a]
2c	–176.0	–192.8	–140.9	–222.3	–299.3

[a] Not detected.

Table 2

^{15}N NMR Parameters of Selected N^7/N^9 Isomers of Substituted Adenines and Guanines

Compound	N-3	N-7	N-9
3a (ref. [11])	–156.8	–142.2	–216.2
3b (ref. [11])	–138.5	–222.8	–137.6
4a (ref. [12])	–198.9	–132.5	–225.1
4b (ref. [12])	–180.6	–216.1	–131.2

chemical shifts δ_H and δ_C are referenced to the middle signal of multiplet of the solvent (δ_H = 2.55 ppm, δ_C = 39.6 ppm).

The assignment of individual signals was carried out on the basis of H-H COSY, gs ^{1}H-^{13}C HSQC and gs ^{1}H-^{13}C HMBC spectra. The carbon spectra were measured both in usual way and by the APT pulse sequence.

^{15}N NMR spectra were measured at 36.50 and 50.69 MHz, respectively. The spectra were measured by both direct detection and by means of the gradient selected ^{1}H-^{15}N HMBC technique. The standardization was carried out with the use of neat nitromethane $CH_3{}^{15}NO_2$ (δ = 0.0 ppm) placed in coaxial capillary. Delays for long-range couplings were set to 10 ms and to 120 ms.

Syntheses of Substances.

1-(4-Vinylbenzyl)uracil (**1a**) and 1-(4-vinylbenzyl)thymine (**1b**): A solution of uracil (2.25 g; 20 mmol) or thymine (2.50 g; 20 mmol) in hexamethyldisilazane (13 ml) was treated with trimethylsilyl chloride (1 ml), and the reaction mixture was refluxed under inert atmosphere (argon) for 24 hrs. The excess hexamethyldisilazane was distilled off in vacuum. The raw silylated uracil or thymine was mixed with a mixture of DMF (10 ml), 4-vinylbenzyl chloride (3 ml; 21 mmol), NaI (30 mg; 0.2 mmol) and hydroquinone (10 mg). The reaction mixture was stirred at 80 °C for 8 hrs, whereupon it was poured in water (150 ml) and after 10-min stirring extracted with dichloromethane (2 × 80 ml). The combined extracts were dried over $MgSO_4$, filtered, and concentrated in a vacuum evaporator. The evaporation residue was recrystallized from methanol to give 2.5 g (55%) white crystals of **1a**, melting at 185-187 °C or 2.2 g (48%) white crystals of **1b**, melting at 162-165 °C. ^{1}H NMR(**1a**): 4.90 (s, 2H, H-7), 5.30 (dd, J 0.3 Hz, J 10.9 Hz, 1H, H-13b), 5.65 (dd, J 2.2 Hz, J 7.8 Hz, 1H, H-5), 5.86 (dd, J 0.3 Hz, J 17.7 Hz, 1H, H-13a), 6.76 (dd, J 17.7 Hz, J 10.9 Hz, 1H, H-12), 7.31-7.32 (m, 2H, H-9), 7.50-7.51 (m, 2H, H-10), 7.80 (d, J 7.8 Hz, 1H, H-6), 11.39 (bs, 1H, H-3). ^{13}C NMR (**1a**): 50.2 (C-7), 101.5 (C-5), 114.7 (C-

13), 126.6 (C-10), 127.9 (C-9), 136.3 (C-12), 136.6 (C-11), 136.8 (C-8), 145.8 (C-6), 151.2 (C-2), 163.9 (C-4).

Anal. Calcd. for $C_{13}H_{12}N_2O_2$: (228.3): C, 68.41; H, 5.30; N, 12.27. Found: C, 68.28; H, 5.22; N, 12.18.

^{1}H NMR (**1b**): 1.80 (d, J 1 Hz, 3H, CH_3), 4.87 (s, 2H, H-7), 5.30 (d, J 10.9 Hz, 1H, H-13b), 5.86 (d, J 17.7 Hz, 1H, H-13a), 6.76 (dd, J 11 Hz, J 17.7 Hz, 1H, H-12), 7.31-7.33 (m, 2H, H-9), .49 (m, 2H, H-10), 7.67 (d, J 1.2 Hz, 1H, H-6), 11.39 (bs, 1H, H-3). ^{13}C NMR (**1b**): 12.1 (CH_3), 49.9 (C-7), 109.2 (C-5), 114.6 (C-13), 126.5 (C-10), 127.9 (C-9), 136.3 (C-12), 136.7 (C-8), 136.8 (C-11), 141.4 (C-6), 151.1 (C-2), 164.4 (C-4).

Anal. Calcd. for $C_{14}H_{14}N_2O_2$: (242.3) C, 69.41; H, 5.82; N, 11.56. Found: C, 69.47; H, 5.33; N, 11.31.

N-4-Acetyl-1-(4-vinylbenzyl)cytosine (**1c**).

A solution of *N*-4-acetylcytosine (3.06 g; 20 mmol) in hexamethyldisilazane (13 ml) was treated with trimythylsilyl chloride (1 ml), and the reaction mixture was refluxed under inert atmosphere (argon) for 24 hrs. The excess hexamethyldisilazane was distilled of in vacuum. The raw *O*,*N*-silylated *N*-4-acetylcytosine was mixed with a mixture of DMF (10 ml), 4-vinylbenzyl chloride (3 ml; 21 mmol), NaI (30 mg; 0.2 mmol), and hydroquinone (10 mg). The reaction mixture was stirred at 80 °C for 16 hrs, whereupon it was poured in water (200 ml), stirred for another 10 min, and extracted with dichloromethane (4 × 100 ml). The combined extracts were dried over $MgSO_4$, filtered, and concentrated in a vacuum evaporator. The evaporation residue was recrystallized from methanol to give 2.7 g (50%) **1c** melting at 114-116 °C. ^{1}H NMR (**1c**): 2.06 (s, 3H, CH_3), 4.96 (s, 2H, H-7), 5.22 (d, J 11 Hz, 1H H-13b), 5.78 (d, J 17.7 Hz, 1H H-13a), 6.68 (dd, J 10.9 Hz, J 17.7 Hz, 1H H-12), 7.15 (d, J 7.2 Hz, 1H H-5), 7.24-7.26 (m, 2H, H-10), 7.41-7.42 (m, 2H, H-9), 8.19 (d, J 7.3 Hz, 1H, H-6), 10.83 (bs, 1H, NH). ^{13}C NMR(**1c**): 24.5 (CH_3), 52.2 (C-7), 95.5 (C-5), 114.7 (C-13), 126.5 (C-10), 12.2 (C-9), 136.3 (C-12), 136.6 (C-8), 136.7 (C-11), 150.4 (C-6), 155.4 (C-2), 162.6 (C-4), 171.1 (C=O).

Anal. Calcd. for $C_{15}H_{15}N_3O_2$: (269.3): C, 69.90; H, 5.61; N, 15.60. Found: C, 69.81; H, 5.55; N, 15.48.

1-(4-Vinylbenzyl)cytosine (**1d**).

A methanolic solution of **1c** (1 g; 4 mmol) in 1.8 M NH_3 (50 ml) was stirred at room temperature overnight. The mixture was evaporated on a water bath until dry, the evaporation residue was washed with water (30 ml) and recrystallized from methanol to give 700 mg (77%) **1d** as white crystals melting at 287-290 °C. ^{1}H NMR (**1d**): 4.88 (, 2H, H-7), 5.29 (d, J 11 Hz, 1H, H-13b), 5.72 (d, J 7.2 Hz, 1H, H-5), 5.85 (d, J 17.7 Hz, 1H, H-13a), 6.75 (dd, J 10.9 Hz, J 17.7 Hz, 1H, H-12), 7.10 (b, 2H, NH_2), 7.27-7.29 (m, 2H, H-9), 7.47-7.49 (m, 2H, H-10), 7.72 (d, J 7.2 Hz, 1H, H-6). ^{13}C NMR (**1d**): 51.1 (C-7), 93.8 (C-5), 114.4 (C-13), 126.3 (C-10), 127.9 (C-9), 136.4 (C-12), 136.4 (C-11), 137.8 (C-8), 14.1 (C-6), 155.9 (C-4), 166.1 (C-2).

Anal. Calcd. for $C_{13}H_{13}N_3O$: (227.3): C, 68.71; H, 5.77; N, 18.49. Found: C, 68.82; H, 5.82; N, 18.37.

9-(4-Vinylbenzyl)adenine (**2a**).

A suspension of adenine (6.75 g; 50 mmol), anhydrous potassium carbonate (7.60 g; 55 mmol), NaI (90 mg; 0.6 mmol), and hydroquinone (10 mg) in DMF (100 ml) was stirred under inert atmosphere (argon) and treated with 4-vinylbenzyl chloride (7.1 ml; 50 mmol). The mixture was refluxed with exclusion of air moisture for 16 hrs, whereupon it was hot filtered, and the solid was washed with hot DMF (2 × 50 ml). The combined filtrates were evaporated in vacuum until dry. The evaporation residue was mixed with chloroform (100 ml) and filtered with silica gel (30 g). The filtrate was concentrated to a volume of 30 ml and, while hot, treated with methanol (30 ml). The crystals separated on cooling were collected by suction and recrystallized from methanol with addition of charcoal. Yield 4.35 g (35%) **2a**, m.p. 221-224 °C. ^{1}H NMR (**2a**): 5.29 (d J 10.9 Hz, 1H, H-16b), 5.40 (s, 2H, H-10), 5.85 (d, J 17.7 Hz, H-16a), 6.74 (dd, J 10.9 Hz, J 17.7 Hz, 1H, H-15), 7.30 (bs, 2H, NH_2), 7.33-7.34 (m, 2H, H-12), 7.47-7.49 (m, 2H, H-13), 8.19 (s, 1H, H-2), 8.30 (s, 1H, H-8). ^{13}C NMR (**2a**): 46.0 (C-10), 114.7 (C-16), 118.8 (C-5), 126.5 (C-13), 128.0 (C-12), 136.2 (C-15), 136.7 (C-14), 136.8 (C-11), 140.9 (C-8), 149.6 (C-4), 152.8 (C-2), 156.1 (C-6).

Anal. Calcd. for $C_{14}H_{13}N_5$: (251.3): C, 66.92; H, 5.21; N, 27.87. Found: C, 67.11; H, 5.10; N, 28.05.

2-Amino-9-(4-vinylbenzyl)-6-chloro-9-*H*-purine (**2b**).

A suspension of 2-amino-7-(4-vinylbenzyl)-6-chloro-9*H*-purine (4 g; 24 mmol), anhydrous potassium carbonate (3.6 g; 26 mmol), and hydroquinone (10 mg) in DMF (50 ml) was stirred under inert atmosphere (argon) and treated with 4-vinylbenzyl chloride (3.4 ml; 12 mmol). The mixture was refluxed with exclusion of air moisture for 32 hrs, whereupon it was hot filtered. The filtrate was evaporated to dryness in vacuum. The evaporation residue was separated by flash chromatography on silica gel (100 g) using a $CHCl_3$-CH_3OH (20:1) mixture as eluent. The first fraction was evaporated until dry, and the residue was recrystallized from a benzene-$CHCl_3$ (1:1) mixture to give 2.25 g (33%) **2b** melting at 193-196 °C. ^{1}H NMR (**2b**): 5.29 (d, J 11 Hz, 1H, H-16b), 5.33 (s, 2H, H-10), 5.85 (d, J 17.6 Hz, 1H, H-16a), 6.74 (dd, J 17.7 Hz, J 10.9 Hz, 1H, H-15), 7.00 (bs, 2H, NH_2), 7.28-7.29 (m, 2H, H-12), 7.48-7.50 (m, 2H, H-13), 8.29 (s, 1H, H-8).

Anal. Calcd. for $C_{14}H_{12}ClN_5$: (285.7): C, 58.85; H, 4.23; N, 24.51; Cl, 12.41. Found: C, 58.98; H, 4.37; N, 24.66; Cl, 12.22.

9-(4-Vinylbenzyl)guanine (**2c**).

A suspension of **2b** (2 g; 7 mmol) in 1 M NaOH (50 ml) was stirred at room temperature overnight. After neutralisation with HCl (1:1) (pH ~ 7), the raw product was collected by suction, dried, and recrystallized from methanol to give 1.2 g (64%) **2c** melting at 186-189 °C. ^{1}H NMR (**2c**): 5.27-5.30 (m, 3H, H-10, H-16b), 5.84 (d, J 17.7 Hz, 1H, H-16a), 6.49 (bs, 2H, NH_2), 6.73 (dd, J 10.9 Hz, J 17.7 Hz, 1H, H-15), 7.23-7.25 (m, 2H, H-12), 7.46-7.48 (2H, H-13), 7.99 (s, 1H, H-8). ^{13}C NMR (**2c**): 45.7 (C-10), 113.8 (C-5), 114.6 (C-16), 126.5 (C-13), 127.5 (C-12), 136.2 (C-15), 136.6 (C-14), 137.0 (C-11), 139.9 (C-8), 154.2 (C-4), 160.1 (C-2), 160.8 (C-6).

Anal. Calcd. for $C_{14}H_{13}N_5O$: (267.3): C, 62.91; H, 4.90; N, 26.20 Found: C, 63.11; H, 4.98; N, 26.11.

Acknowledgments.

The authors wish to acknowledge the financial support provided by the Max-Planck Society Germany and Ministry of Education, Youth and Sports of the Czech Republic (Project CI MSM 253 100 001).

REFERENCES AND NOTES

[1] N. V. Rothwell, In Understanding Genetics: A Molecular Approach, Wiley-Liss: New York, 1993; Capters 11 and 12.

[2] A. Khan, D. M. Haddelton, M. J. Hannon, D. Kukulj and A.

Marsh, *Macromolecules*, **32**,6560 (1999).

[3] A. Marsh, A. Khan, D. M. Haddelton and M. J. Hannon, *Macromolecules*, **32**, 8725 (1999).

[4] L. De Napoli, A. Messere, D. Montesarchio, G.Piccialli, C. Santacroce and G. M. Bonora, *Nucleosides Nucleotides*, **12**, 23 (1993).

[5] A. Loregian, R. Gratti, G. Palu and E. F. De Palo, *J. Chromatogr.,B*, **764**, 289 (2001).

[6] N. Baret, J. P. Dulcere, J. Rodriquez, J. M. Pons and R. Faure, *Eur. J. Org. Chem.*, 1507 (2000).

[7] A. Holý ,*Coll. Czech. Chem. Commun.*,**46**, 3134 (1981).

[8] G. Langli, L. L. Gundersen and F. Rise, *Tetrahedron*, **15**, 5625 (1996).

[9] Z. Timár, L. Kovács, G.Kovács and Z. Schmél, *J. Chem. Soc. Perkin Trans. 1*, 19 (2000).

[10] D. Hocková, M. Buděšínský, R. Marek, J. Marek and A. Holý, *Eur. J. Org. Chem.*, 2675 (1999).

[11] G. Remaud, J. Kjellberg, H. Bazin, N. G. Johansson and J. Chattopadhyaya, *Tetrahedron*, **42**, 5073 (1986).

[12] R. Marek, J. Brus, J. Toušek, L. Kovács and D. Hocková, *Magn. Reson. Chem*, **40**, 353 (2002).

[13] V. Markowski, G. R. Sullivan and J. D. Roberts, *J. Am. Chem. Soc.*, **99**, 714 (1977).

[14] M. Witanowski, L. Stefaniak and G. A.Webb, Annu. Rep. NMR Spect.Vol. **25**; Academic Press: San Diego, 1993, pp 322.

[15] S. G. Srivatsan, S. Verma and M. Parvez, *Acta Crystallogr., Sec. C: Cryst. Struct. Commun.*, 378 (2002).