A REGIOSELECTIVE SYNTHESIS OF ALKYL 2-(GUANIN-9-YL)ACETATES AS PNA BUILDING BLOCKS FROM 7-(4-NITROBENZYL)GUANINE DERIVATIVES

György Ferenc[1a], Péter Forgó[b], Zoltán Kele[2a] and Lajos Kovács[3a,*]

[a] *Department of Medicinal Chemistry, University of Szeged, Dóm tér 8, H-6720 Szeged, Hungary; e-mail: [1] gyorgyi@ovrisc.mdche.u-szeged.hu, [2] kele@ovrisc.mdche.u-szeged.hu, [3] kovacs@ovrisc.mdche.u-szeged.hu*

[b] *Department of Organic Chemistry, University of Szeged, Dóm tér 8, H-6720 Szeged, Hungary; e-mail: pforgo@chem.u-szeged.hu*

Received July 29, 2004
Accepted October 8, 2004

Guanine derivatives substituted at N^7 with 4-R-benzyl groups (R = H, MeO, NO_2) have been evaluated in the regioselective N^9-alkylation of guanine. Given the capricious removal of (substituted) benzyl groups from guanine derivatives and pent-4-enoylation of guaninium hydrochloride, an improved alternative approach has been elaborated consisting in the pent-4-enoylation and per-*O*-acetylation of guanosine (**8**), 4-nitrobenzylation at N^7 followed by *N*-glycoside hydrolysis (**10**), N^9-alkylation (**13**) and deprotection with sodium dithionite to afford the peptide nucleic acid building block *tert*-butyl [N^2-(pent-4-enoyl)guanin-9-yl]-acetate (**15**) in 36% overall yield. This avoids N^7 regioisomer formation, solubility problems and any chromatographic purification. A remarkable influence of the *O*- and/or N^2-acyl groups on the stability of *N*-glycosidic bond and reactivity of 2-amino group was observed. The structure of a pyrimidine by-product **12** arising from the imidazole ring-opening of guaninium salt **4d** in alkaline medium has been elucidated by 2D NMR.

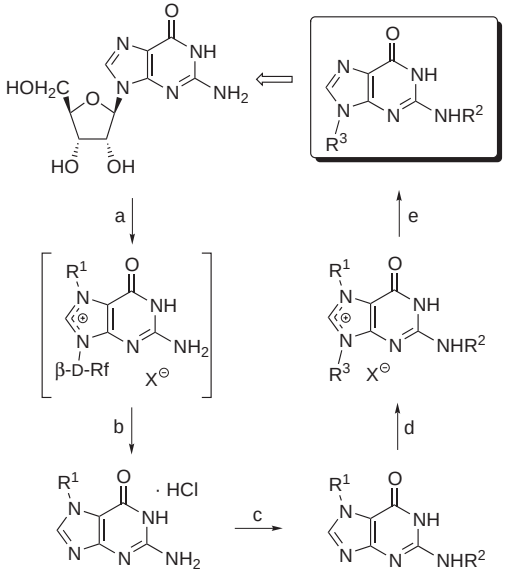
Keywords: Guanine; Regioselective alkylation; Guanosine; Nucleoside analogues; Nucleosides; Purines; Peptide nucleic acids.

The regioselective alkylation of guanine at N^9 is an important synthetic route to pharmaceutically important acyclic nucleoside analogues (e.g. acyclovir, ganciclovir, HPMPG) and monomeric building blocks of oligonucleotide analogues (e.g. peptide nucleic acids, PNA); however these reactions are rarely regiospecific, leading to mixtures of 9- and 7-alkylated products which can be very difficult to separate[1].

The regioselectivity and yield in the synthesis of 7- or 9-substituted guanosine analogues can be high in the alkylation of persilylated guanine derivatives e.g. O^6-(*N*,*N*-diphenylcarbamoyl)-N^2-isobutyrylguanine with β-*O*-activated alkylation reagents[1,2] e.g. peracylated sugars[3]. 9-Alkoxyalkyl-

ated products can be obtained in high yields even if an N^9/N^7 isomeric mixture is formed while the N^7 to N^9 rearrangement takes place upon heating[4–10] or even at room temperature in DMF [11].

The regioselectivity of the alkylation with non-β-*O*-activated, small-sized alkyl halides (e.g. alkyl haloacetates in the synthesis of PNA monomers) under basic conditions (e.g. K_2CO_3) is still inadequate. Constraining guanine into its lactim form e.g. in the case of 2-amino-6-chloropurine[12–17], 2-amino-6-(arylsulfanyl)purines[18] or O^6-(*N*,*N*-diphenylcarbamoyl) derivatives[19] improves the N^9/N^7 isomer ratio, but it is not sufficient requirement since the highest yield of N^9-isomer was around 75% and chromatographic purification could not be avoided in every case, furthermore 2-amino-6-chloropurine is mutagenic and expensive, 2-amino-6-(arylsulfanyl)purines require a strong acidic treatment, the *N*,*N*-diphenylcarbamoyl group can be labile[20,21] and this imposes limitations on its applicability. There is a third approach, however, affording exclusively 9-alkylated derivatives. Izawa et al. reported an N^9-regioselective substitution starting from guanosine[22,23]. In their approach guanosine was protected on N^7 with a (substituted) benzyl group; then, after acid hydrolysis of *N*-glycosidic bond and



SCHEME 1

The reaction sequence followed by Izawa et al.[22,23] Reagents and conditions: a R^1X, b HCl, c R^2X, d R^3X, e removal of R^1. β-D-Rf = β-D-ribofuranosyl, R^1 = substituted benzyl, R^2 = acyl, R^3 = alkyl

acylation of the amino group, 7-substituted benzyl-N^2-acylguanines were alkylated selectively at N^9, then deprotected at N^7 in a good overall yield (ca. 80%, Scheme 1). In this case the (substituted) benzyl is not only a protective group but it activates the purine ring in alkylation[22,23].

RESULTS AND DISCUSSION

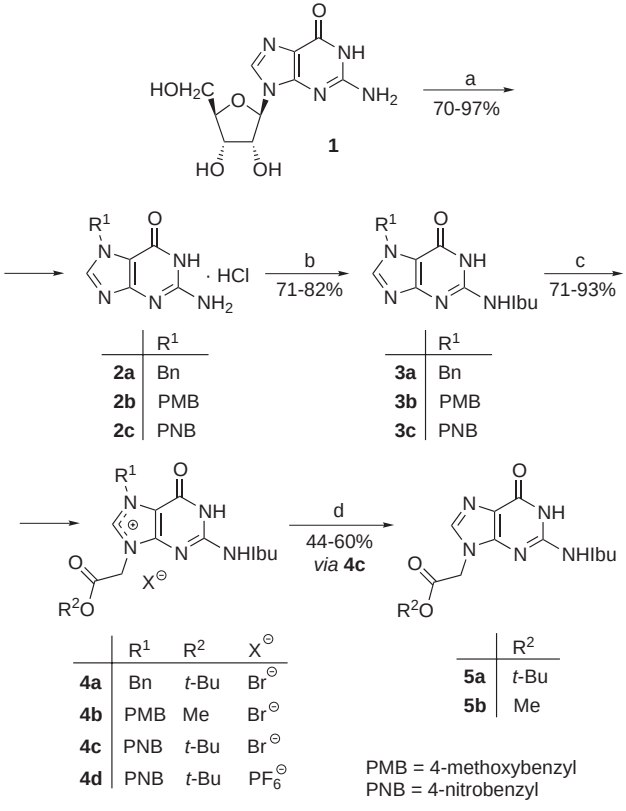
The problem of the regioselectivity of the alkylation of guanine derivatives was present in the reaction of *tert*-butyl bromoacetate with N^2-isobutyrylguanine and O^6-(*N*,*N*-diphenylcarbamoyl)-N^2-isobutyrylguanine as the first step in the synthesis of Fmoc/acyl-protected PNA monomers[24]. In our initial study the Izawa method has been adopted for the regioselective N^9-alkylation of guanine. Thus, 7-benzylguanine hydrochloride[25] (**2a**), available from guanosine (**1**) in a one-pot procedure, was acylated with isobutyric anhydride and the resulting compound **3a** was allowed to react with *tert*-butyl bromoacetate (Scheme 2). The guaninium salt **4a** was subjected to hydrogenolysis to remove the benzyl group giving rise to ester **5a**. All the steps afforded crystalline compounds, there was no need for chromatographic purification and the overall yield of the four-step process, starting from guanosine, was 37%. However, the last step often proved to be capricious and irreproducible. The notorious behavior of benzylated purines under hydrogenolysis is well documented[26] and alternative *N*-debenzylation with the potassium *tert*-butoxide–dimethyl sufoxide–oxygen system is not compatible with the vulnerable, base-sensitive groups[27,28]. Consequently, we decided to abandon the benzyl group.

The 4-methoxybenzyl (PMB) group is known to be cleaved from ethers or amines under oxidative conditions[29] thus the above reaction was repeated (Scheme 2) employing this group with the only difference being the application of methyl bromoacetate as alkylating agent (the corresponding *tert*-butyl ester was not crystalline). The intermediates **2b**–**4b** were again nice crystalline compounds and the transformations were uneventful until the last step. The removal of the PMB group from **4b** under oxidative (cerium ammonium nitrate[30], DDQ [31], potassium peroxodisulfate[32]), catalytic[33] or transfer hydrogenolytic[34] or acid ($AlCl_3$-anisole[35,36], CBr_4-methanol[37]) conditions has not been successful. The PMB group in the guaninium moiety of compound **4b** proved to be very resistant to the above reagents.

The reductive deprotection of 4-nitrobenzyl (PNB) group is a well-established method, therefore we have embarked upon the synthesis and use of 7-(4-nitrobenzyl)guanines. The reaction sequence carried out with

benzyl and PMB groups has been repeated with 4-nitrobenzyl bromide and standard transformations led to guaninium salt **4c** (Scheme 2). The removal of PNB group has been accomplished with Zn/AcOH or In/AcOH and ester **5a** has been obtained from guanosine in 38–40% overall yield; however, the last step required chromatographic purification and removal of zinc acetate and the yellow by-product was cumbersome.

In our experience the removal of guanine isobutyryl group was not quantitative in the cleavage and deprotection of PNA oligomers[38]. Therefore a change in the protective group was needed. Pent-4-enoyl group seems to be a better choice since it can be removed, in addition to the conventional ammonia, with iodine as well[39–41].



	R^1
2a	Bn
2b	PMB
2c	PNB

	R^1
3a	Bn
3b	PMB
3c	PNB

	R^1	R^2	$X^{\ominus}$
4a	Bn	*t*-Bu	$Br^{\ominus}$
4b	PMB	Me	$Br^{\ominus}$
4c	PNB	*t*-Bu	$Br^{\ominus}$
4d	PNB	*t*-Bu	$PF_6^{\ominus}$

	R^2
5a	*t*-Bu
5b	Me

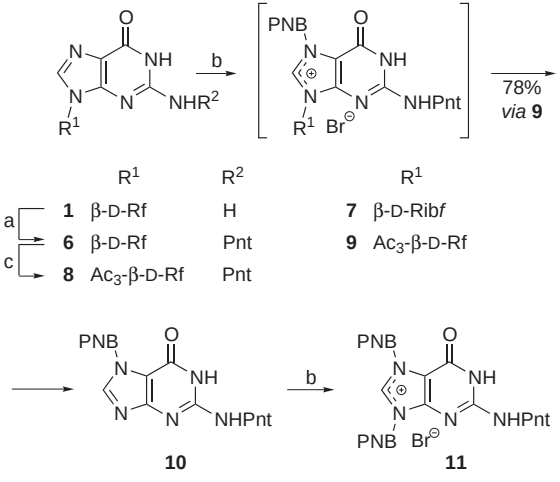
SCHEME 2

Reagents and conditions: a 1. R^1Br, DMSO; 2. HCl, then MeOH. b Ibu_2O, Et_3N, DMF, 150 °C, 3 h. c $BrCH_2COOR^2$, DMF, 60 °C, 24 h. d Zn, aq. AcOH, r.t., 18 h

7-(4-Nitrobenzyl)guanine hydrochloride (**2c**) did not dissolve well during the pent-4-enoylation, hence another route was needed to protect the 2-amino group. Considering the high price of pent-4-enoic anhydride, the reactivity of the amino group and the solubility of compounds the sequence N^2-acylation, N^7-protection, *N*-glycoside hydrolysis, N^9-alkylation seemed to be the only good choice.

N^2-(Pent-4-enoyl)guanosine (**6**) was obtained in a high yield by temporary protection of hydroxy groups with the electron-donating trimethylsilyl group[40,42]. The 7,9-bis(4-nitrobenzyl)guaninium salt **11**, detected in the reaction mixture by TLC/MS, was the product in subsequent reaction with 4-nitrobenzyl bromide (Scheme 3), 7-(4-nitrobenzyl)-N^2-(pent-4-enoyl)guanine (**10**) could not even be detected. The formation of 7,9-bis(arylmethyl)guanines under forced conditions (elevated temperatures and prolonged reaction times) has been reported[43].

Enhancing the stability of the *N*-glycosidic bond is imperative for the monoalkylation of guanosine derivatives by introduction of electron-withdrawing, e.g. acetyl groups to the hydroxy groups of the saccharide moiety. Pent-4-enoylation of 2′,3′,5′-tri-*O*-acetylguanosine was a slow and low-yielding process even at higher temperature, therefore 2′,3′,5′-tri-*O*-



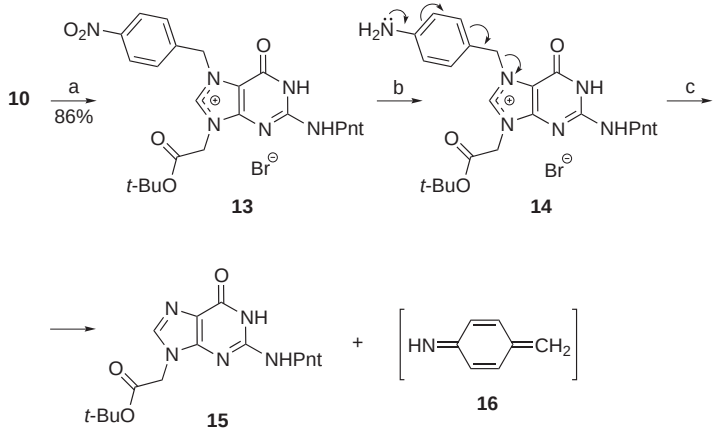
SCHEME 3

Reagents and conditions: a 1. TMSCl, pyridine, r.t., 1 h; 2. pent-4-enoic anhydride, pyridine, r.t., 16 h; 3. water, 0–5 °C, 5 min; 4. aq. NH_3, r.t., 30 min. b 1–10 equiv. 4-nitrobenzyl bromide, DMF, r.t., up to 6 days, see the text. c Ac_2O, pyridine, DMF, r.t., 16 h. a + c 71%. β-D-Rf = β-D-ribofuranosyl, Ac_3-β-D-Rf = 2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl

acetyl-N^2-(pent-4-enoyl)guanosine (**8**) was synthesized by acetylation of N^2-(pent-4-enoyl)guanosine (**6**). The formation of guaninium salt **9** was slow but compound **11** formed only in a negligible amount. The reaction was complete after 2.5 days with an usual 4-equivalent excess of the reagent. The excess of 4-nitrobenzyl bromide was scavenged by pyridine in order to avoid the formation of the dialkylated product **11** during evaporation of the solvent (DMF). 4-Nitrobenzylpyridinium bromide and compound **10** was separated with extraction after thermolysis of the *N*-glycoside taking place at 70 °C without acid treatment.

The extent of the influence of the *O*- and/or N^2-acyl groups on the stability of *N*-glycosidic bond and the reactivity of N^2 was notable in the above reactions. 7-(4-Nitrobenzyl)guanosinium ion was stable but it decomposed spontaneously in the absence of acid after acylation on N^2. Acetylation of hydroxy groups in compound **7** stabilized the *N*-glycosidic bond at room temperature but thermolysis took place without acid at 70 °C. The acetyl groups in 2′,3′,5′-tri-*O*-acetylguanosine withdrew the electron density of the purine ring and, at the same time, from the 2-amino group to such an extent that the acylation was not complete even after a prolonged time at 100 °C. On the other hand, trimethylsilyl group activated the 2-amino group and the acylation took place smoothly.

Alkylation of compound **10** affording guaninium salt **13** (Scheme 4), with 3 equivalents of *tert*-butyl bromoacetate at 70 °C was complete overnight.



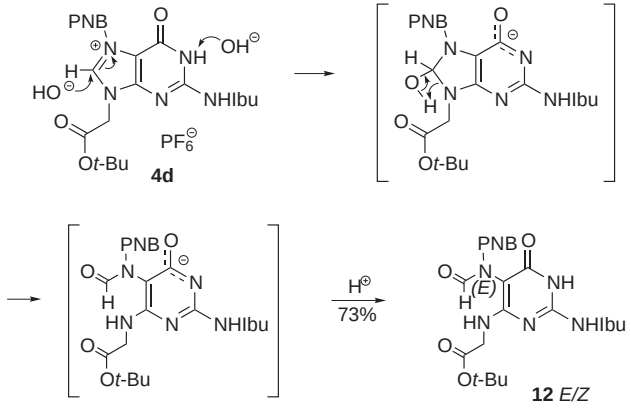
SCHEME 4

Reagents and conditions: a 3 equiv. *tert*-butyl bromoacetate, DMF, 70 °C, 16 h; b 4 equiv. $Na_2S_2O_4$, aq. acetone, pH 7.0, r.t., 30 min; c 70 °C, 16 h; b + c 76%

As mentioned earlier, the purification of ester **5a** after PNB removal with Zn/acetic acid was too laborious, therefore other reducing agents have been studied.

Sodium sulfide was successfully used for the deprotection of 4-nitrobenzyl esters and carbamates[44] and our initial studies were conducted with salt **4d**. In the sodium sulfide treatment, a new substance **16** (Scheme 5) emerged, the polarity of which was similar to the expected product **5a**. However, in its MS spectrum the molecular ion $[M + H]^+$ was observed at *m/z* 489 instead of at the expected value *m/z* 336. Imidazole ring opening is known to occur in alkaline solution as C-8 is electrophilic in salts[45] like **4d**. The exact position of formyl group (N^4 or N^5) has not been ascertained earlier[46] or only unconvincingly proved[47]. Our 2D NMR measurements (HSQC, HMBC) have unequivocally demonstrated (Fig. 1) that the formyl group is located on N^5 confirming that the ensuing ring scission takes place between C-8 and N-9 atoms of guaninium salt **4d**. Compound **12** exists as a 7:1 mixture of rotamers at room temperature in DMSO-d_6 solution.

Reduction of the nitro group in compound **13** was complete within 30 min in the presence of 4 equivalents of sodium dithionite even at pH 4, not only at pH 8–9 where deprotection of 4-nitrobenzyloxycarbonyl group was achieved[44]. To enhance the rearrangement of 4-aminobenzyl group leading to the deprotection, the reaction mixture was heated at 70 °C in a phosphate buffer (pH 7) and acetone was employed to obtain a homogenous solution (the intermediacy of **14** was verified by TLC/MS, Scheme 4).



SCHEME 5
Imidazole ring-opening of guaninium salt **4d** in alkaline solution

Based on the observation that electron-donating substituents on phenyl ring and/or on α-carbon atom promote the rearrangement by stabilizing the positive charge on benzyl methylene group in the removal of 4-nitrobenzyl carbamates[48], it was not suprising that heating of the reaction mixture was required for the deprotection as, in our case, a positively charged substituent was attached to the benzyl methylene group decreasing the stability of the formed cation.

Iminoquinomethane **16** was the elusive by-product of this deprotection regime. This compound has never been isolated in pure form due to its instability and tendency to polymerize[48–50]. We have been able to detect

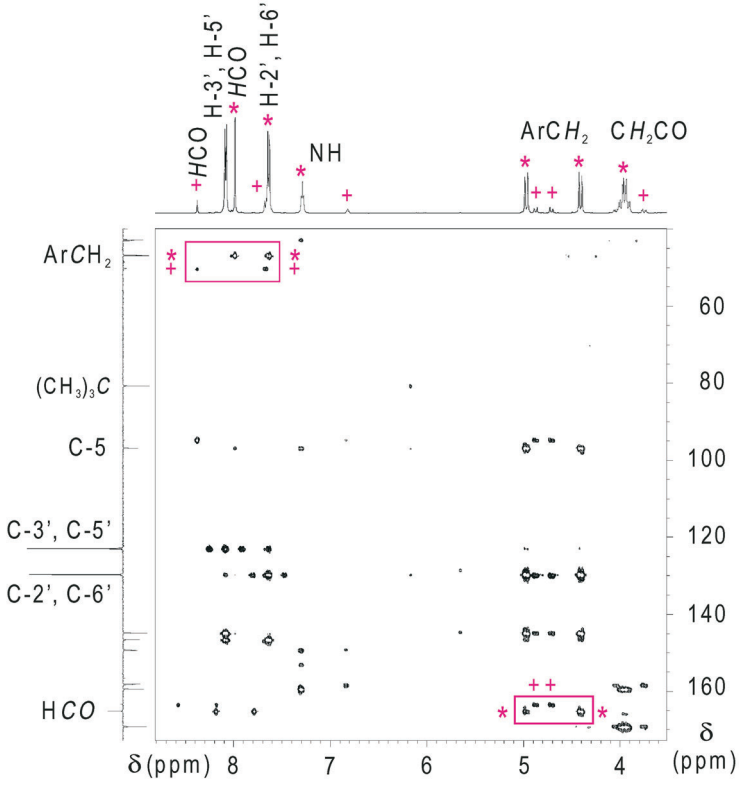


FIG. 1

HMBC spectrum (detail) of compound **12**. Crucial correlations are highlighted by boxes. For pyrimidine numbering, see Scheme 5, primed numbers denote atoms in the benzene ring. Some signals are doubled in the major (*) and minor (+) rotamers, e.g. the formyl protons at 7.99 (*) and 8.38 ppm (+), respectively

in the ESI mass spectrum peaks of this substance at *m/z* 106 and *m/z* 138 corresponding to ions $[M + H]^+$ and $[M + MeOH + H]^+$, respectively, but our further attempts to characterize this compound have not been rewarding. The presence of this substance as a contaminant was obvious in both the yellow-colored aqueous and the organic phases. It was attempted to scavenge the substance with sulfites[49] (oxidized form of dithionite), however, it was impossible to completely remove this substance from the product. The slightly yellowish amorphous product **15** was easily obtained by washing with ethyl acetate; it was completely pure according to TLC and NMR analyses.

The site of the alkylation (N^9 vs N^7) in guanine derivatives can be unequivocally ascertained by ^{1}H [18,51,52], ^{13}C [24,51,52] and ^{15}N NMR [53] or MS/MS [54] methods. In this study our ^{13}C NMR method[24] was used. The structures of

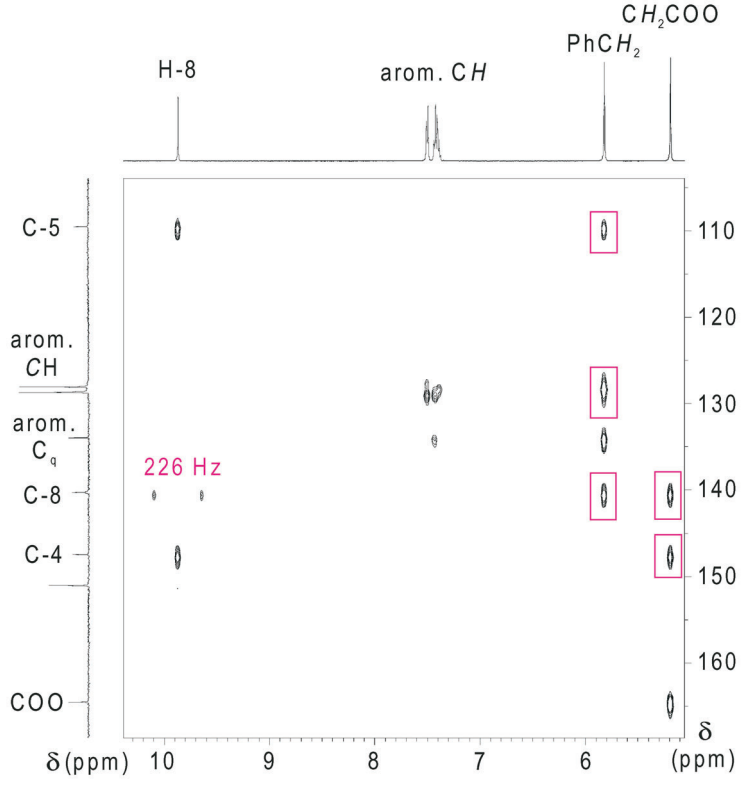
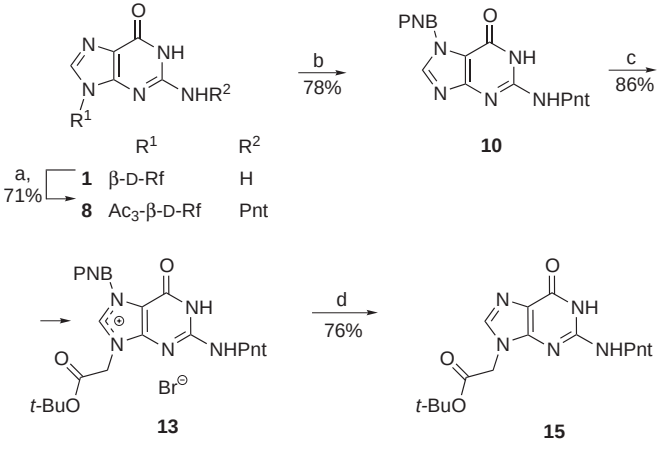


FIG. 2

HMBC spectrum (detail) of compound **4a**. Crucial correlations are highlighted by boxes. For purine numbering, see Scheme 1

compounds **3**, **10** and **15** were corroborated by the chemical shift difference parameters $a = \delta_{C\text{-}4} - \delta_{C\text{-}5}$, $b = \delta_{C\text{-}8} - \delta_{C\text{-}5}$ and $c = \delta_{C\text{-}5} - \delta_{C\text{-}1'}$, involving C-1′, C-4, C-5 and C-8 which are the most sensitive to the site of alkylation (data not shown). It is noteworthy that the guaninium salts display an unusually large heteronuclear coupling constant between H-8 and C-8 (e.g. $^1J_{H\text{-}8,C\text{-}8}$ = 226 (**4a**) and 227 Hz (**4c**)), which indicates the imidazolium substructure[55–57]. The structure of compound **4a** has been corroborated by HSQC and HMBC investigations as well (Fig. 2).

In our study directed to 9-substituted guanines, the application of guanine derivatives substituted at N^7 with different benzyl groups (benzyl, 4-methoxybenzyl, 4-nitrobenzyl) has been evaluated. The general sequence exemplified in Schemes 1 and 2 proved to be problematic in terms of easy and reproducible removal of protecting group at N^7 and acylation of compounds **2** with pent-4-enoic anhydride. Our improved alternative approach described in this paper comprises the following steps: (i) pent-4-enoylation of guanosine at N^2 (**6**), (ii) per-*O*-acetylation (**8**), (iii) 4-nitrobenzylation at N^7 (**9**), (iv) hydrolysis of the *N*-glycoside **9** (**10**), (v) N^9-alkylation (**13**) and (vi) deprotection with sodium dithionite. This seemingly lengthy procedure can be combined into four distinct, well-reproducible steps with standard transformations affording the PNA building block *tert*-butyl [N^2-(pent-



SCHEME 6

Reagents and conditions: a 1. TMSCl, pyridine, r.t., 1 h; 2. 1.25 equiv. pent-4-enoic anhydride, pyridine, r.t., 16 h; 3. water, 0–5 °C, 5 min; 4. aq. NH_3, r.t., 30 min; 5. Ac_2O, pyridine, DMF, r.t., 16 h. b 4 equiv. 4-nitrobenzyl bromide, DMF, r.t., 60 h. c 3 equiv. *tert*-butyl bromoacetate, DMF, 70 °C, 16 h. d 1. 4 equiv. $Na_2S_2O_4$, aq. acetone, pH 7.0, r.t., 30 min; 2. 70 °C, 16 h. β-D-Rf = β-D-ribofuranosyl, Ac_3-β-D-Rf = 2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl

4-enoyl)guanin-9-yl]acetate (**15**) with no N^7 regioisomer formation, solubility problems or chromatographic purification (Scheme 6) with the same 36% overall yield as in the case of Izawa et al. method. Main advantages of our synthesis are the acylation of N^2 amino group at room temperature due to the high solubility of the starting compound and a reproducible removal of the 4-nitrobenzyl group. Attempts at the conversion of **15** to a guanine PNA monomer are under way. The extent of the influence of the *O*- and/or N^2-acyl groups on the stability of *N*-glycosidic bond and reactivity of 2-amino group is remarkable. The site of the alkylation in 7- and 9-alkylated guanine derivatives has been corroborated by chemical shift differences of ^{13}C NMR spectra. Position of the formyl group (N^5) in the ring-opened derivative **12** formed from guaninium salt **4d** under alkaline conditions (Scheme 5) has been unravelled by 2D NMR.

EXPERIMENTAL

The following abbreviations are employed: Bn (benzyl), *t*-Bu (*tert*-butyl), TMSCl (chlorotrimethylsilane), DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone), Et_2O (diethyl ether), DMF (*N*,*N*-dimethylformamide), DMSO (dimethyl sulfoxide), ESI (electrospray ionization), EtOAc (ethyl acetate), Fmoc (fluoren-9-ylmethoxycarbonyl), Ibu (isobutyryl), MeOH (methanol), PMB (4-methoxybenzyl), PNB (4-nitrobenzyl), Pnt (pent-4-enoyl), PNA (peptide nucleic acid(s)), r.t. (room temperature), TEA (triethylamine).

Chemicals were purchased from Aldrich, Fluka, Merck or Reanal (Budapest, Hungary). Pent-4-enoic anhydride was alternatively synthesized from pent-4-enoic acid as described in literature[58,59]. Compound **2a** was prepared as described[25]. Compounds **2b** and **2c** were prepared in an analogous way[60]. 4-Methoxybenzyl bromide is prone to decomposition and it was freshly prepared prior to use from the corresponding alcohol[61]. Anhydrous solvents were prepared as described[62]. Organic solutions were dried using anhydrous $MgSO_4$ and evaporated in Büchi rotary evaporators. TLC: Kieselgel 60 F_{254} (Merck); solvent systems: CH_2Cl_2–MeOH 9:1 (S1), CH_2Cl_2–MeOH 95:5 (S2), CH_2Cl_2–iPrOH 5:0.25 (S3); visualization: UV light, H_2SO_4/ethanol. All guaninium salts gave oval spots with significant tailing. M.p.: Electrothermal IA 8103 apparatus. Elemental analysis: Perkin–Elmer CHN analyzer model 2400. UV: PE Lambda 10 spectrometer, λ_{max} in nm (log ε); sh, shoulder. Stock solutions were made in ethanol except of compounds **3b** and **10**, in which cases adding DMSO was necessary to achieve clear solutions. In the case of latter samples and TEAc buffer the region below 220 nm in UV spectra was uncertain, because these compounds have high absorbance in this region. NMR: Bruker Avance DRX 500 spectrometer (^1H: 500.13 MHz, ^{13}C: 125.76 MHz), DMSO-d_6 solutions; δ (ppm), *J* (Hz). Spectral patterns: s, singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet; br, broad; deut, deuterable. The superscripts *, # denote interchangeable assignments. For the 2D experiments (HMQC, HMBC), standard Bruker software packages (INV4GSSW, INV4GSLRNDSW) were used. Mass spectrometry: Finnigan MAT TSQ 7000, electrospray (ESI) technique. TLC/MS: the analyte solution has been applied onto a 5 cm wide silica gel TLC plate as a band to obtain sufficient material. After developing in a solvent system, the appropriate band was scraped off with a spatula, the silica gel was

suspended in MeOH (100 µl), sonicated, centrifuged and the supernatant was used for MS analysis.

7-Benzyl-N^2-isobutyrylguanine (**3a**)

Compound[25] **2a** (8.393 g, 30.2 mmol) suspended in anhydrous DMF (90 ml) was treated with isobutyric anhydride (12.50 ml, 75.5 mmol) and TEA (8.43 ml, 60.4 mmol) and the mixture was heated with stirring at 150 °C for 3 h. The homogeneous solution was evaporated in vacuo, coevaporated with MeOH (2×), triturated under cold water, filtered and dried. The crude product (8.913 g) was boiled with ethanol (200 ml), the obtained solution filtered and the filtrate was evaporated to dryness. Trituration under cold ether gave the title compound (6.663 g, 70.8%) as beige crystals, m.p. 176–178 °C. R_F 0.65 (S1). For $C_{16}H_{17}N_5O_2$ (311.3) calculated: 61.7% C, 5.5% H, 22.5% N; found: 61.55% C, 5.4% H, 22.7% N. UV: λ_{max} (50% (v/v) 1 M HCl in EtOH, pH 0) 202 sh (4.22), 267 (3.98); λ_{max} (50% (v/v) 1 M TEAc in EtOH, pH 7) 267 (3.97); λ_{max} (50% (v/v) 0.1 M KOH in EtOH, pH 13) 272 (3.82). ^{1}H NMR: 1.12 d, 6 H, J = 6.8 ((C**H**$_3$)$_2$CH); 2.75 pseudo t, 1 H, J = 6.8 ((CH$_3$)$_2$C**H**); 5.52 s, 2 H (CH$_2$); 7.29–7.34 m, 5 H (C$_6$H$_5$); 8.33 s, 1 H (H-8); 11.49 s, 1 H (NH); 12.13 s, 1 H (NH). ^{13}C NMR (J-mod. spin-echo): 18.65 ((**C**H$_3$)$_2$CH); 34.52 ((CH$_3$)$_2$**C**H); 49.08 (Ph**C**H$_2$); 110.99 (C-5); 127.32, 127.64, 128.45 (arom. CHs); 137.09 (arom. C$_q$); 144.08 (C-8); 147.00, 152.49, 157.07 (C-2, C-4, C-6); 179.75 (iPr**C**O). MS (ESI), *m/z* (rel.%): 623 (30) [2 M + H]$^+$), 312 (100) [M + H]$^+$.

N^2-Isobutyryl-7-(4-methoxybenzyl)guanine (**3b**)

Prepared from 7-(4-methoxybenzyl)guanine hydrochloride[60] (**2b**; 7.8 mmol scale) as described for compound **3a** (1.989 g, 74.7%). An analytical sample was obtained by recrystallizing the product from acetonitrile (4.6 g/100 ml), m.p. 185.9–186.6 °C (acetonitrile). R_F 0.48 (S1). For $C_{17}H_{19}N_5O_3$ (341.4) calculated: 59.8% C, 5.6% H, 20.5% N; found: 59.6% C, 5.65% H, 20.3% N. UV: λ_{max} (50% (v/v) 1 M HCl in EtOH, pH 0) 223 (4.13), 267 (4.08); λ_{max} (50% (v/v) 1 M TEAc in EtOH, pH 7) 267 (4.00); λ_{max} (50% (v/v) 0.1 M KOH in EtOH, pH 13) 271 (3.90). ^{1}H NMR: 1.10 d, 6 H, J = 6.8 ((C**H**$_3$)$_2$CH); 2.74 pseudo t, 1 H, J = 6.8 ((CH$_3$)$_2$C**H**); 3.71 s, 3 H (CH$_3$O); 5.42 s, 2 H (CH$_2$); 6.88 d, 2 H, J = 8.5 (ArH); 7.33 d, 2 H, J = 8.5 (ArH); 8.31 s, 1 H (H-8); 11.51 s, 1 H (NH); 12.13 s, 1 H (NH). ^{13}C NMR (J-modulated spin-echo): 18.75 ((**C**H$_3$)$_2$CH); 34.60 ((CH$_3$)$_2$**C**H); 48.66 (Ar**C**H$_2$); 54.98 (CH$_3$O); 110.92 (C-5); 113.92 (C-3′, C-5′); 129.12, 129.17 (C-2′, C-6′, C-1′); 143.97 (C-8); 147.02, 152.57, 157.18, 158.86 (C-2, C-4, C-6, C-4′); 179.84 (iPr**C**O). MS (ESI), *m/z* (rel.%): 683 (32) [2 M + H]$^+$, 342 (100) [M + H]$^+$.

N^2-Isobutyryl-7-(4-nitrobenzyl)guanine (**3c**)

Prepared from 7-(4-nitrobenzyl)guanine hydrochloride[60] (**2c**; 8.6 mmol scale) as described for compound **3a** (2.512 g, 81.8%). The substance was sufficiently pure for further transformations, however, it can be recrystallized from MeOH (2.0 g/150 ml), m.p. > 260 °C. R_F 0.68 (S1). For $C_{16}H_{16}N_6O_4$ (356.3) calculated: 53.9% C, 4.5% H, 23.6% N; found: 53.75% C, 4.6% H, 23.4% N. UV: λ_{max} (50% (v/v) 1 M HCl in EtOH, pH 0) 201 (4.22), 218 (4.05), 267 (4.11); λ_{max} (50% (v/v) 1 M TEAc in EtOH, pH 7) 267 (4.15); λ_{max} (50% (v/v) 0.1 M KOH in EtOH, pH 13) 272 (4.11). ^{1}H NMR: 1.10 d, 6 H, J = 6.7 ((C**H**$_3$)$_2$CH); 2.72 pseudo t, 1 H, J = 6.7 ((CH$_3$)$_2$C**H**); 5.66 s, 2 H (CH$_2$); 7.53 d, 2 H, J = 8.3 (ArH); 8.18 d, 2 H, J = 8.3 (ArH); 8.38 s, 1 H (H-8); 11.55 s, 1 H (NH); 12.13 s, 1 H (NH). ^{13}C NMR (J-modulated spin-echo): 18.77

((CH_3)$_2$CH); 34.64 ((CH_3)$_2$CH); 48.61 (ArCH_2); 111.06 (C-5); 123.72, 128.43 (arom. CHs); 144.51 (C-8); 144.64, 146.97, 147.29, 152.51, 157.36 (C-2, C-4, C-6, C-1′, C-4′); 179.92 (iPrCO). MS (ESI), *m/z* (rel.%): 713 (5) $[2\ M + H]^+$, 357 (100) $[M + H]^+$.

7-Benzyl-9-[(*tert*-butoxycarbonyl)methyl]-N^2-isobutyrylguaninium Bromide (**4a**)

Compound **3a** (1.557 g, 5.0 mmol) dissolved in warm DMF (20 ml) was allowed to react with *tert*-butyl bromoacetate (0.813 ml, 5.5 mmol) at 60 °C for 24 h. The solution was evaporated in vacuo, coevaporated with EtOAc (3×) and EtOAc was added (20 ml). The resulting oil slowly crystallized in a refrigerator (1.801 g, 71%), m.p. 158 °C (dec., EtOAc). R_F ca. 0.2 (S1). For $C_{22}H_{28}BrN_5O_4$ (506.4) calculated: 52.2% C, 5.6% H, 13.8% N; found: 52.0% C, 5.7% H, 13.65% N. UV: λ_{max} (50% (v/v) 1 M HCl in EtOH, pH 0) 201 (4.26), 271 (3.97); λ_{max} (50% (v/v) 1 M TEAc in EtOH, pH 7) 278 (3.70); λ_{max} (50% (v/v) 0.1 M KOH in EtOH, pH 13) 243 (3.96). ^{1}H NMR: 1.12 d, 6 H, *J* = 6.9 ((CH_3)$_2$CH); 1.44 s, 9 H (*t*-Bu); 4.02 pseudo t, 1 H, *J* = 6.9 ((CH_3)$_2$C**H**); 5.19 s, 2 H (CH_2COO); 5.82 s, 2 H (PhC**H**$_2$); 7.39–7.50 m, 5 H (C_6H_5); 9.87 s, 1 H (H-8); 12.10 br s, 1 H (NH); 12.70 s, 1 H (NH). ^{13}C NMR (*J*-modulated spin-echo, HSQC, HMBC experiments): 18.56 ((CH_3)$_2$CH); 27.48 ((CH_3)$_3$C); 34.72 ((CH_3)$_2$CH); 46.40 (CH_2COO); 51.65 (PhCH_2); 83.57 ((CH_3)$_3$C); 109.61 (C-5); 128.16, 128.80 (arom. CHs); 134.04 (arom. C_q); 140.28 (C-8); 147.55 (C-4); 151.13 (C-2, C-6); 164.62 (COO); 180.52 (iPrCO). MS (ESI), *m/z* (rel.%): 426 (100) M^+, guaninium ion.

N^2-Isobutyryl-7-(4-methoxybenzyl)-9-[(methoxycarbonyl)methyl]guaninium Bromide (**4b**)

Prepared from compound **3b** (5.33 mmol) as described for compound **4a** (2.382 g, 90%), m.p. 147.3–150.6 °C (EtOAc). R_F ca. 0.2 (S1). For $C_{20}H_{24}BrN_5O_5$ (494.3) calculated: 48.6% C, 4.9% H, 14.2% N; found: 48.55% C, 5.0% H, 14.35% N. UV: λ_{max} (50% (v/v) 1 M HCl in EtOH, pH 0) 200 sh (4.45), 270 (4.02); λ_{max} (50% (v/v) 1 M TEAc in EtOH, pH 7) 273 (3.83); λ_{max} (50% (v/v) 0.1 M KOH in EtOH, pH 13) 273 (3.80). ^{1}H NMR: 1.11 d, 6 H, *J* = 6.7 ((CH_3)$_2$CH); 2.80 pseudoquintet, 1 H, *J* = 6.7 ((CH_3)$_2$C**H**); 3.74 and 3.76 each s, 6 H 2 × (CH_3O); 5.30 s, 2 H (CH_2COO); 5.72 s, 2 H (ArC**H**$_2$); 6.97 d, 2 H, *J* = 8.3 (ArH); 7.49 d, 2 H, *J* = 8.3 (ArH); 9.77 s, 1 H (H-8); 12.05 br s, 1 H (NH); 12.60 s, 1 H (NH). ^{13}C NMR (*J*-modulated spin-echo): 18.50 ((CH_3)$_2$CH); 34.70 ((CH_3)$_2$CH); 45.89 (CH_2COO); 51.36 (ArCH_2); 52.98, 55.13 (2 × CH_3O); 109.63 (C-5); 114.21, 130.19 (arom. CHs); 125.56 (C-1′*); 141.03 (C-8); 147.60 (C-4′*); 151.04, 151.10, 159.64 (C-2, C-4, C-6); 166.23 (COO); 180.49 (iPrCO). MS (ESI), *m/z* (rel.%): 414 (100) M^+, guaninium ion.

9-[(*tert*-Butoxycarbonyl)methyl]-N^2-isobutyryl-7-(4-nitrobenzyl)guaninium Bromide (**4c**) and Hexafluorophosphate (**4d**)

Prepared from compound **3c** (10.0 mmol) as described for compound **4a**. The bromide crystallized very sluggishly. It was very well soluble in EtOAc, therefore a water-insoluble hexafluorophosphate salt (**4d**) was prepared as follows. The crude oily product from the above reaction was dissolved in acetonitrile (20 ml) and ammonium hexafluorophosphate (1.793 g, 11.0 mmol) in water (10 ml) was added. The solution was evaporated, the residue was triturated under cold water and the precipitate was stored in vacuo over P_2O_5 (5.732 g, 93.0%). The title hexafluorophosphate dissolves well in cold MeOH, EtOAc, CH_2Cl_2 and acetonitrile but not in Et_2O or water. The salt can be precipitated from acetonitrile solution by adding water. M.p. 126.8–127.5 °C (aqueous acetonitrile). R_F (**4c**) ca. 0.10 (S1). For

$C_{22}H_{27}F_6N_6O_6P$ (**4d**; 616.4) calculated: 42.9% C, 4.4% H, 13.6% N; found: 42.65% C, 4.35% H, 13.4% N. UV (**4c**): λ_{max} (50% (v/v) 1 M HCl in EtOH, pH 0) 200 sh (4.50), 270 (4.31); λ_{max} (50% (v/v) 1 M TEAc in EtOH, pH 7) 271 (4.08); λ_{max} (50% (v/v) 0.1 M KOH in EtOH, pH 13) 245 (4.11), 269 (4.06). ^{1}H NMR (**4c**): 1.10 d, 6 H, *J* = 6.7 ((CH_3)$_2$CH); 1.45 s, 9 H (*t*-Bu); 2.81 pseudoquintet, 1 H, *J* = 6.7 ((CH_3)$_2$C**H**); 5.22 s, 2 H (CH_2COO); 5.99 s, 2 H (ArCH_2); 7.73 d, 2 H, *J* = 8.3 (ArH); 8.24 d, 2 H, *J* = 8.3 (ArH); 9.95 s, 1 H (H-8); 12.10 br s, deut, 1 H (NH); 12.50 br s, deut, 1 H (NH). ^{13}C NMR (**4c**; *J*-modulated spin-echo): 18.61 ((CH_3)$_2$CH); 27.55 ((CH_3)$_3$C); 34.77 ((CH_3)$_2$CH); 46.54 (CH_2COO); 50.99 (ArCH_2); 83.68 ((CH_3)$_3$C); 109.72 (C-5); 123.76, 129.29 (arom. CHs); 141.42, 147.52, 147.68, 151.04, 151.21 (C-1′, C-4′, C-2, C-4, C-6, C-8); 164.64 (COO); 180.58 (iPrCO). MS (ESI) (**4c**), *m/z* (rel.%): 471 (100) M^+, guaninium ion, 941 (10) $[2\ M - H^+]^+$.

tert-Butyl (*N*^{2}-Isobutyrylguanin-9-yl)acetate[24] (**5a**)

Compound **4c** (0.240 g, 0.435 mmol) was dissolved in 50% (v/v) acetic acid (5 ml), zinc powder (0.178 g, 2.72 mmol, 6.0 equivalents) was added and the mixture was stirred at room temperature for 18 h. A further portion of zinc powder (0.090 g) was added and stirring was continued for 6 h. The zinc almost completely dissolved and a yellow precipitate was formed. After filtration through a Hyflo bed and thorough washing with acetonitrile, the solution was evaporated and dissolved in a mixture of dichloromethane (25 ml) and water (25 ml). The aqueous phase was extracted with dichloromethane (25 ml) and the combined organic phases were washed with 0.05 M EDTA disodium salt (2 × 10 ml), dried and evaporated in vacuo (0.077 g). The Hyflo bed was washed again with acetonitrile and a combined crop of 0.130 g was obtained. Chromatography (CH_2Cl_2–MeOH 98:2 to 94:6) yielded 0.064 g (43.8%), m.p. 203 °C. R_F 0.13 (S2). The NMR and MS data of the product were in full agreement with those previously published[24]. In another experiment after the work-up as above, the resulting red oil was coevaporated with acetonitrile (3×) and the yellow solid obtained (0.82 g, 60%) showed a single spot on TLC. Further trituration under ether yielded a purer product (0.60 g, 44%).

2′,3′,5′-Tri-*O*-acetyl-*N*^{2}-(pent-4-enoyl)guanosine (**8**)

Guanosine hydrate (8.8 g, 31.1 mmol) was suspended in acetonitrile (2 × 100 ml) and evaporated to dryness. Chlorotrimethylsilane (30 ml, 234 mmol) was added dropwise (20 min) to the suspension of dried guanosine in anhydrous pyridine (150 ml) and stirred for another 40 min. Pent-4-enoic anhydride (7.10 ml, 38.9 mmol, 1.25 equivalents) was added and the reaction was stirred at room temperature for 16 h. The cooled reaction mixture was diluted with water (30 ml) and treated with ammonia solution (30 ml, 25%) for 30 min. The residue was dissolved in water (400 ml) and extracted with a mixture of Et_2O and EtOAc (1:1 v/v, 400 ml). The water phase was evaporated, then coevaporated with acetonitrile (2 × 300 ml) and used for the next step without further purification.

2-(Pent-4-enoyl)guanosine (**6**) was dissolved in a mixture of DMF (44 ml) and pyridine (22 ml). Acetic anhydride (18 ml) was added to the mixture, the pyridinium salts from the previous step were filtered off and the solution was set aside for 16 h. Ethanol (10 ml) was added to the solution, then the residue was dissolved in EtOAc (400 ml) and extracted with 1 M hydrochloric acid (2 × 300 ml) and saturated $NaHCO_3$ solution (2 × 300 ml). Evaporation in vacuo after drying gave the product as a white solid foam (10.9 g, 71%). R_F 0.30 (S2),

0.45 (S3). UV: λ_{max} (50% (v/v) 1 M HCl in EtOH, pH 0) 202 (4.20), 260 (3.97), 281 (3.84); λ_{max} (50% (v/v) 1 M TEAc in EtOH, pH 7) 260 (4.00), 281 (3.87); λ_{max} (50% (v/v) 0.1 M KOH in EtOH, pH 13) 266 (3.97). ^{1}H NMR: 2.03 s, 3 H (CH_3CO); 2.04 s, 3 H (CH_3CO); 2.11 s, 3 H (CH_3CO); 2.34 m, 2 H ($CH_2CH=CH_2$); 2.58 t, 2 H, *J* = 7.2 (CH_2CO); 4.29 m, 1 H (H-4′); 4.37 m, 2 H (H-5′); 4.99 d, 1 H, *J* = 10.2 (*cis*-CH_2=CH); 5.06 d, 1 H, *J* = 17.2 (*trans*-CH_2=CH); 5.48 dd, 1 H, *J* = 5.7, *J* = 3.7 (H-3′); 5.81 m, 2 H (H-1′, CH_2=CH); 6.08 d, 1 H, *J* = 6.3 (H-2′); 8.23 s, 1 H (H-8); 11.60 br s, 1 H (NH); 12.06 br s, 1 H (NH). ^{13}C NMR (decoupled and *J*-modulated spin-echo spectra): 20.03, 20.25, 20.39 (3 × CH_3CO); 28.07 ($CH_2CH=CH_2$); 35.10 (CH_2CO); 63.00 (C-5′); 70.28 (C-3′*); 72.16 (C-2′*); 79.83 (C-4′*); 84.56 (C-1′); 115.54 (CH_2=CH); 120.37 (C-5); 136.73 (CH_2=CH); 137.74 (C-8); 148.06 (C-2$^{\#}$); 148.57 (C-6$^{\#}$); 154.65 (C-4); 169.15, 169.35, 169.99 (3 × CH_3CO); 175.46 (C_4H_7CONH). MS (ESI), *m/z* (rel.%): 492.16 (100) $[M + H]^+$.

7-(4-Nitrobenzyl)-N^2-(pent-4-enoyl)guanine (**10**)

Compound **8** (4.9 g, 10.0 mmol) dissolved in anhydrous DMF (60 ml) and 4-nitrobenzyl bromide (8.6 g, 40.0 mmol) were stirred at room temperature for 60 h. When the reaction was complete, pyridine (6.4 ml, 80 mmol) was added to scavenge excess of the alkylation reagent and set aside for 5 h. The reaction mixture was heated at 70 °C for 16 h to thermolyse the guaninium salt **9**. The solution was evaporated in vacuo and EtOAc (400 ml) and water (400 ml) were added to the oily residue. The product (2.3 g, 62%) was precipitated and filtered off. A further crop (0.6 g, 16%) was precipitated when the residue from the evaporated organic phase was treated with CH_2Cl_2 (10 ml). Overall yield of **10**: 2.9 g (78%), amorphous solid. R_F 0.29 (S2), 0.22 (S3). UV: λ_{max} (50% (v/v) 1 M HCl in EtOH, pH 0) 267 (4.15); λ_{max} (50% (v/v) 1 M TEAc in EtOH, pH 7) 267 (4.14); λ_{max} (50% (v/v) 0.1 M KOH in EtOH, pH 13) 272 (4.11). ^{1}H NMR: 2.32 m, 2 H ($CH_2CH=CH_2$); 2.53 t, 2 H, *J* = 7.2 (CH_2CO); 4.97 d, 1 H, *J* = 10.2 (*cis*-CH_2=CH); 5.04 d, 1 H, *J* = 17.1 (*trans*-CH_2=CH); 5.65 s, 2 H ($ArCH_2$); 5.77–5.85 m, 1 H (CH_2=C**H**CH_2); 7.52 d, 2 H, *J* = 8.5 (ArH); 8.17 d, 2 H, *J* = 8.5 (ArH); 8.37 s, 1 H (H-8); 11.57 br s, 1 H (NH); 12.08 br s, 1 H (NH). ^{13}C NMR (decoupled and *J*-modulated spin-echo spectra): 28.13 (CH_2=$CHCH_2$); 34.96 (CH_2CO); 48.60 ($ArCH_2$); 111.08 (C-5); 115.49 (CH_2=CH); 123.68, 128.44 (arom. CH); 136.73 (CH_2=CH); 144.49, 144.54, 147.00, 152.47, 157.33 (C-8, C-1′, C-4′, C-2, C-6, C-4); 175.26 (C_4H_7CONH). MS (ESI), *m/z* (rel.%): 368.97 (100) $[M + H]^+$.

tert-Butyl ({2-Isobutyramido)-5-[*N*-(4-nitrobenzyl)formamido]-6-oxo-1,6-dihydropyrimidin-4-yl}amino)acetate (**12**)

To compound **4d** (0.308 g, 0.5 mmol) dissolved in acetone (3 ml) was added sodium sulfide nonahydrate (0.48 g, 2.0 mmol) in water (1 ml) and the mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with water (20 ml) and extracted with CH_2Cl_2 (3 × 10 ml), the organic layer was dried and evaporated (0.178 g, 73%), m.p. 191 °C (dec.). R_F 0.39 (S2). For $C_{22}H_{28}N_6O_7$ (488.5) calculated: 54.1% C, 5.8% H, 17.2% N; found: 53.9% C, 5.95% H, 16.95% N. UV: λ_{max} (50% (v/v) 1 M HCl in EtOH, pH 0) 201 (4.24), 232 (4.42), 276 (4.00); λ_{max} (50% (v/v) 1 M TEAc in EtOH, pH 7) 276 (3.94); λ_{max} (50% (v/v) 0.1 M KOH in EtOH, pH 13) 245 (4.09), 270 (4.02). ^{1}H NMR (HSQC, HMBC, see Fig. 1, major rotamer): 1.06 d, 6 H, *J* = 6.5 ($(CH_3)_2CH$); 1.37 s, 9 H (*t*-Bu); 2.73 pseudoquintet, 1 H, *J* = 6.5 ($(CH_3)_2CH$); 3.96 m, 2 H (CH_2COO); 4.41, 4.97 2 × d, 2 × 1 H, *J* = 15.2 ($ArCH_2$); 7.29 t, 1 H, *J* = 6.0 (NH); 7.64 d, 2 H, *J* = 8.5 (ArH); 7.99 s, 1 H (HCO); 8.08 d, 2 H, *J* = 8.5 (ArH);

11.30 br s, 1 H (NH); 11.41 br s, 1 H (NH). ^{13}C NMR (HSQC, HMBC, see Fig. 1, major rotamer): 18.62 ($(CH_3)_2CH$); 27.65 ($(CH_3)_3C$); 34.58 ($(CH_3)_2CH$); 42.90 (CH_2COO); 46.82 ($ArCH_2$); 80.74 ($(CH_3)_3C$); 96.83 (C-5); 122.95 (C-3′, C-5′); 129.74 (C-2′, C-6′); 144.90 (C-1′); 146.60 (C-4′); 149.36 (C-2*); 158.15 (C-6*); 159.42 (C-4); 165.14 (HCO); 169.20 (COO*t*-Bu); 180.36 (iPrCO). MS (ESI), *m/z* (rel.%): 977.6 (48) $[2 M + H]^+$, 489 (100) $[M + H]^+$.

9-[(*tert*-Butoxycarbonyl)methyl-7-(4-nitrobenzyl)-N^2-(pent-4-enoyl)guaninium Bromide (**13**)

tert-Butyl bromoacetate (1.2 ml, 8.1 mmol) was added to a solution of compound **10** (1.0 g, 2.7 mmol) in anhydrous DMF (40 ml) and heated at 70 °C for 16 h. The residue obtained after evaporation was dissolved in a phosphate buffer (60 ml, pH 7.0) and extracted with Et_2O (60 ml). The title salt **13** (1.30 g, 86%) precipitated as a white amorphous solid. R_F 0.21 (S1). UV: λ_{max} (50% (v/v) 1 M HCl in EtOH, pH 0) 201 sh (4.28), 270 (4.14); λ_{max} (50% (v/v) 1 M TEAc in EtOH, pH 7) 272 (4.02); λ_{max} (50% (v/v) 0.1 M KOH in EtOH, pH 13) 245 (4.02), 269 (3.98). ^{1}H NMR: 1.41 s, 9 H ($(CH_3)_3C$); 2.30 br s, 2 H ($CH_2CH=CH_2$); 2.63 br s, 2 H (CH_2CO); 4.95 d, 1 H, *J* = 8.3 (*cis*-CH_2=CH); 5.02 d, 1 H, *J* = 16.8 (*trans*-CH_2=CH); 5.15 s, 2 H (CH_2COO); 5.81 br s, 1 H ($CH=CH_2$); 5.96 s, 2 H ($ArCH_2$); 7.72 d, 2 H, *J* = 7.9 (ArH); 8.19 d, 2 H, *J* = 7.9 (ArH); 9.81 s, 1 H (H-8); 11.32 br s, 1 H (NH); 12.20 br s, 1 H (NH). ^{13}C NMR (decoupled and *J*-modulated spin-echo spectra): 27.45 ($C(CH_3)_3$); 28.22 ($CH_2CH=CH_2$); 35.29 (CH_2CONH); 45.99 (CH_2COO); 50.36 ($ArCH_2$); 83.27 ($C(CH_3)_3$); 109.50 (C-5); 115.12 (CH_2=CH); 123.62 (ArCH); 129.26 (ArCH); 137.11 (CH_2=CH); 138.99 (C-8); 142.06 (C-1′, C-4′); 147.37 (C-2*); 148.30 (C-6*); 153.83 (C-4); 164.92 (COO*t*-Bu); 173.75 (C_4H_7CONH). MS (ESI), *m/z* (rel.%): 483 (100) $[M^+]$.

tert-Butyl [N^2-(Pent-4-enoyl)guanin-9-yl]acetate (**15**)

Sodium dithionite (0.790 g, 3.6 mmol, 80%) was added to a solution of guaninium salt **13** (0.530 g, 0.94 mmol) in acetone (15 ml) and phosphate buffer (15 ml, pH 7.0). After 30 min stirring at room temperature, the solution was heated at 70 °C for 16 h. Acetone was evaporated in vacuo and the water phase was extracted with EtOAc (50 ml). The title ester **15** (0.250 g, 76%) was obtained as a yellowish amorphous solid. R_F 0.19 (S2), 0.22 (S3). UV: λ_{max} (50% (v/v) 1 M HCl in EtOH, pH 0) 203 (4.22), 261 sh (4.03); λ_{max} (50% (v/v) 1 M TEAc in EtOH, pH 7) 260 (3.98), 280 (3.85); λ_{max} (50% (v/v) 0.1 M KOH in EtOH, pH 13) 267 (3.89). ^{1}H NMR: 1.40 s, 9 H ($C(CH_3)_3$); 2.33 q, 2 H, *J* = 7.0 ($CH_2CH=CH_2$); 2.56 t, 2 H, *J* = 7.2 (CH_2CO); 4.86 s, 2 H (CH_2COO); 4.98 d, 1 H, *J* = 10.1 (*cis*-CH_2=CH); 5.05 d, 1 H, *J* = 16.3 (*trans*-CH_2=CH); 5.82 m, 1 H ($CH=CH_2$); 7.94 s, 1 H (H-8); 11.66 br s, 1 H (NH); 12.04 br s, 1 H (NH). ^{13}C NMR (decoupled and *J*-modulated spin-echo spectra): 27.56 ($(CH_3)_3C$); 28.09 ($CH_2CH=CH_2$); 34.98 (CH_2CO); 44.76 (CH_2COO); 82.24 ($(CH_3)_3C$); 115.49 (CH_2=CH); 119.54 (C-5); 136.74 (CH_2=CH); 140.23 (C-8); 147.76 (C-2*); 148.85 (C-6*); 154.73 (C-4); 166.49 (COO*t*-Bu); 175.50 (C_4H_7CONH). MS (ESI), *m/z* (rel.%): 717.41 (38) $[2 M + Na]^+$, 695.43 (68) $[2 M + H]^+$, 370 (18) $[M + Na]^+$, 348 (100) $[M + H]^+$.

This research has been supported by OTKA (grant T 22551) and EU FP6 (grant Targeting replication and integration of HIV, TRIoH 503480).

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