Regioselective alkylation of guanine derivatives in the synthesis of peptide nucleic acid monomers*

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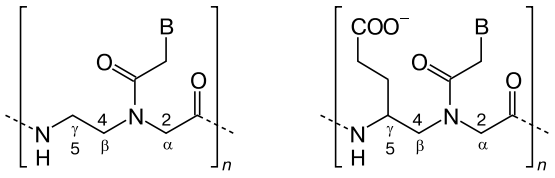
A method for the synthesis of 6-*O*-benzyl- and 6-*O*-benzyl-2-*N*-benzyloxycarbonyl-protected guanine derivatives starting from 2-amino-6-chloropurin is described. A regioselective alkylation of these N(9)-protected guanine derivatives gave the corresponding α-monomers of chiral peptide nucleic acids, the L-glutamic acid derivatives. It was shown that these compound do not inhibit (in the concentrations <20 μmol L^{-1}) the topoisomerase I activity.

Key words: peptide nucleic acids, diprotected guanine, topoisomerase I.

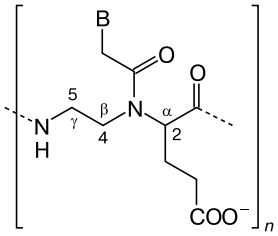
Guanine-containing compounds alkylated at N(9) atom are of special interest. First of all, this is due to the fact that acyclic guanine analogs[1] are widely used in the therapy of viral infections. It is known that 6-*O*-substituted guanine in the DNA composition hinders activity of topoisomerase I,[2] whereas 6-*C*-substituted purins are inhibitors of topoisomerase II activity.[3] In this case, the enzyme serves as a target for antitumor agents, therefore the screening of new compounds on the topoisomerase activity is still important in the present time. Other well known derivatives are the guanine monomers of functional polyamide DNA analogs, the peptide nucleic acids (PNA),[4] which are used in molecular biology and diagnosis.[5]

At the present time, PNA are considered as a new class of stable compounds capable of overcoming a number of disadvantages of natural oligonucleotides (ON) and their analogs when used as genetic therapeutic agents. An *N*-(2-aminoethyl)glycine (aeg) fragment with nucleic heterocyclic bases attached through the acetyl linkers serves as a structural unit of the PNA skeleton. Such a structure, on the one hand, due to the "restrained flexibility"[6] secures an efficient recognition of complimentary sequences by the nucleic bases incorporated in the skeleton,[7] on the other hand, due to the unnatural bonds makes the aeg-PNA stable to nucleases and proteases.[8] Earlier, it was shown that the introduction of a negative charge in the structure of PNA using the phosphate groups[9] did not noticeably decrease the stability of their complexes with ON, as well as facilitates the bioavailability of PNA when using a standard cationic lipofection.[10] The introduction of a substituent at positions 2(α) or 5(γ) of the PNA core leads to the appearance of a chiral center, whose configuration is responsible for the formation of the oligomer preorganized structure, that, in the end, determines the hybridizing properties of PNA.[11] Thus, we consider it a promising direction to develop chiral negatively charged PNA, which, together with nucleic bases responsible for molecular recognition, contain a negative charge per each monomer unit, like in natural ON.



aeg-PNA

γ-PNA



α-PNA

B is Thy, Cyt, Ade, Gua.

* On the occasion of the 100th anniversary of the birth of Academician N. K. Kochetkov (1915—2005).

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Lately, both the classic and the modified PNA are used in the studies of noncanonical structures of nucleic acids[12] in order to determine a regulatory function of these elements in the process of gene expression, as well as in the analysis of telomeric repeats. To study such interactions, it is necessary to obtain PNA oligomers with a large content of guanine bases, which requires a preparative, well reproducible procedure for the synthesis of guanine monomers.

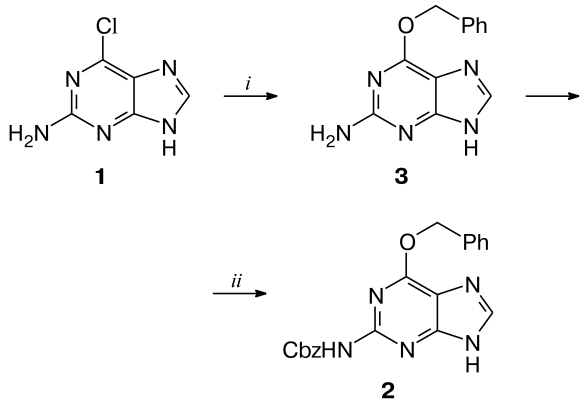
The developed by now strategies for the synthesis of PNA monomers for their subsequent oligomerization,[13—15] suggest either the condensation of a pseudopeptide fragment with a heterocycle carboxymethylated derivative[16] or the alkylation of protected heterocycles with a bromoacetamide derivative[17] of the pseudopeptide fragment. Earlier, it was shown that the second strategy is more optimal for the synthesis of monomers, whose side chain is represented by the 3-benzyloxycarbonyl(ethyl) moiety,[18] since it gives higher yields. An aeg-guanine monomer was obtained by the condensation of a pseudopeptide fragment with 9-*N*-alkyl-6-*O*-benzyl guanine derivative,[16] however, the unprotected exocyclic amino group of the heterocycle makes it impossible to carry out a qualitative Kaiser test and capping the amino groups in the growing oligomeric chain. Later, guanine 2-*N*-benzyloxycarbonyl-9-*N*-carboxymethyl derivative was synthesized,[19] however, in all these cases[16,19] the introduction of protecting groups in the heterocycle was carried out after 9-*N*-alkylation of 2-amino-6-chloropurin **1**. For the synthesis of the PNA monomers through the alkylation of protected heterocycles with chloro(bromo)acetic acid esters,[18] it is also necessary to protect O(6) in order to prevent the side alkylation process at atom N(7). However, it was shown[20] that the alkylation of 2-*N*-isobutiryl-6-*O*-diphenylcarbamoylguanine with methyl bromoacetate led to a mixture of products of N(9)/N(7)-substitution, the content of the 7-*N*-regioisomer being ~15%.

To sum up, to obtain the PNA guanine monomers based on L-Glu, the Boc-protocol of the solid-phase synthesis requires a preliminary protection of the guanine O(6) and N(2) according to the earlier described tactics for the introduction of protecting groups in the synthesis of the thymine monomer.[21]

The diprotected guanine derivative **2** was obtained in two steps. In the first step, a benzyl protecting group was introduced at atom O(6) according to a slightly changed procedure described earlier:[22] sodium hydride was used instead of sodium for the formation of sodium benzylate. The yield of compound **3** was 62%. The reaction of 6-*O*-benzylguanine **3** with benzyloxycarbonyl chloride (CbzCl) in the presence of sodium hydride (Scheme 1) led to guanine derivative **2** in 44% yield.

The starting compound for the synthesis of guanine monomers was the secondary amine **4** (see Ref. 23), which was converted to the corresponding bromoacetamide derivative **5** (Scheme 2). Earlier, this synthesis[24] was accomplished by the addition of bromoacetyl bromide to a mixture of pseudopeptide **4** and Et_3N in dichloromethane. However, when the amount of compound **4** was scaled to 5 g, the yield of bromide **5** was only 43%. Apparently, a side cyclic product **6** is formed[23] upon the initial addition of Et_3N to the secondary amine **4** (see Scheme 2), and this process becomes very fast upon an increase in the loading of amine **4**. To avoid this side reaction, we changed the addition order of the reagents. Thus, bromoacetyl bromide was added to a solution of compound **4** in dichloromethane at 0 °C and only after this Et_3N was added slowly dropwise. The yield of compound **5** with the changed order of addition of reagents was 85%.

Scheme 1



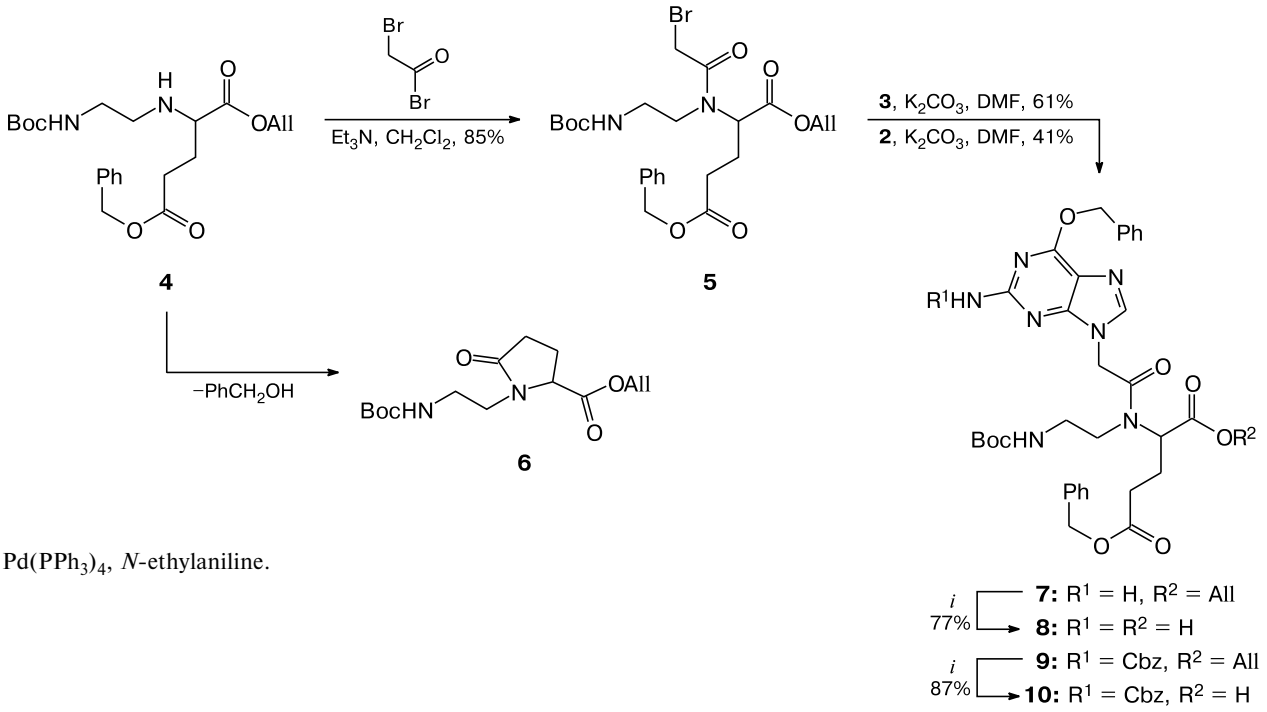
i. $PhCH_2OH$, NaH, 60 °C, 62%; *ii*. CbzCl, NaH, DMF, 44%.

The guanine-containing monomers **7** and **9** protected at the *C*-end were obtained by the alkylation of protected heterocyclic bases **2** and **3** with bromoacetamide derivative **5** in the presence of 1.1—1.2 equiv. of K_2CO_3. Monomer **7** was obtained in 65% yield, whereas a completely protected monomer **9** in 41% yield. The moderate yields can be explained by the incomplete conversion of the starting compounds **2** and **3**, which were separated from the target products by column chromatography.

When evaluating the regioselectivity of the alkylation reaction of purin bases **2** and **3** with bromoacetamide derivative **5**, the N(7)/N(9)-substitution cannot be inferred from the data of ^{13}C NMR spectroscopy because of a possibility of the incorrect interpretation of close signals for the carbon atoms of the allyl protecting group and atom C(5) of the purin bases. Therefore, the regioselectivity of alkylation was studied using 2D NMR spectroscopy ($^1H/^1H$ COSY, $^1H/^{13}C$ HSQC, $^1H/^{13}C$ HMBC) for monomer **7**, since the interpretation of its spectral data is simpler than that for monomer **9**.

During analysis, we assigned the signals for ^{13}C and ^1H nuclei in the NMR spectra of monomer **7**, in which the double sets of signal for all the H and C atoms were observ-

Scheme 2



i. $Pd(PPh_3)_4$, *N*-ethylaniline.

ed caused by the presence of two conformers of compound **7** (Fig. 1) due to the hindered rotation around the amide bond between the AAL and the pseudopeptide fragment.

To determine the regioselectivity of alkylation at atom N(9), we used the HMBC spectra. The analysis of the spectra showed the presence of a correlation between the methylene protons of AAL (δ_H 5.13, a major conformer; δ_H 5.03, a minor conformer) and atoms C(8) (δ_C 142.1) and C(4) (δ_C 156.6) of the guanine fragment of the bases. At the same time, there were no correlation between the linker protons and atom C(5) (δ_C 115.9) of the base, which indicated the presence of the exclusive N(9)-substitution products only (see Fig. 1, *b*). A fragment of the 2D HMBC spectrum of monomer **7** is shown in Fig. 2 with the indication of the correlating signals.

Monomer **9** was not characterized by 2D NMR spectra, however, the regular 1D ^1H and ^{13}C NMR spectra showed similar signals for all the H and C atoms with the additional signals attributed to the Cbz group. The AAL methylene protons are assigned to δ 5.16 (a major conformer) and δ 5.08 (a minor conformer), whereas the signals for atoms C(8), C(4), and C(5) of the guanine fragment are found at δ 140.0, 156.0, and 119.1, respectively. It is known that in the case of N(7)-substitution, the chemical shifts for atoms C(4) and C(5) can be found at δ 157—164 and 108—112, respectively.[20]

To sum up, the alkylation of protected guanine bases **2** and **3** with the pseudopeptide bromoacetamide derivatives **5** proceeds regioselectively and leads to the target products of N(9)-alkylation.

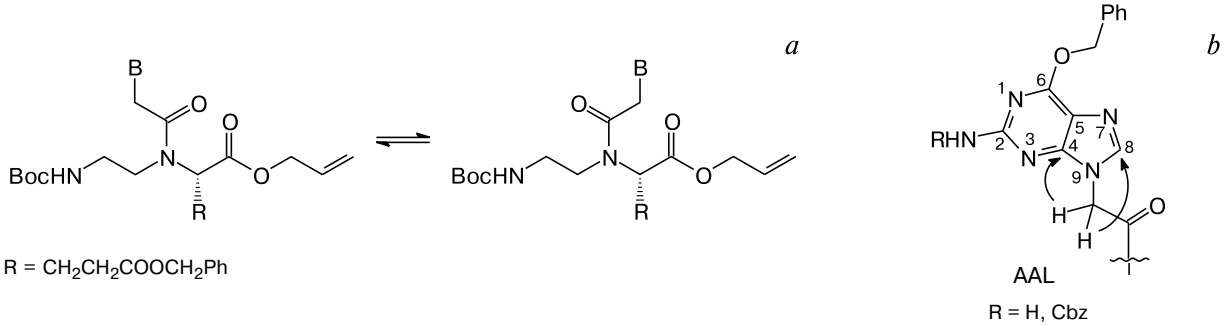


Fig. 1. (*a*) Structures of conformers formed owing to the hindered rotation around the amide bond between the N(3)-monomeric unit and the C(O)-acetamide linker (AAL); (*b*) heteronuclear correlation indicating the regioselectivity of the alkylation reaction.

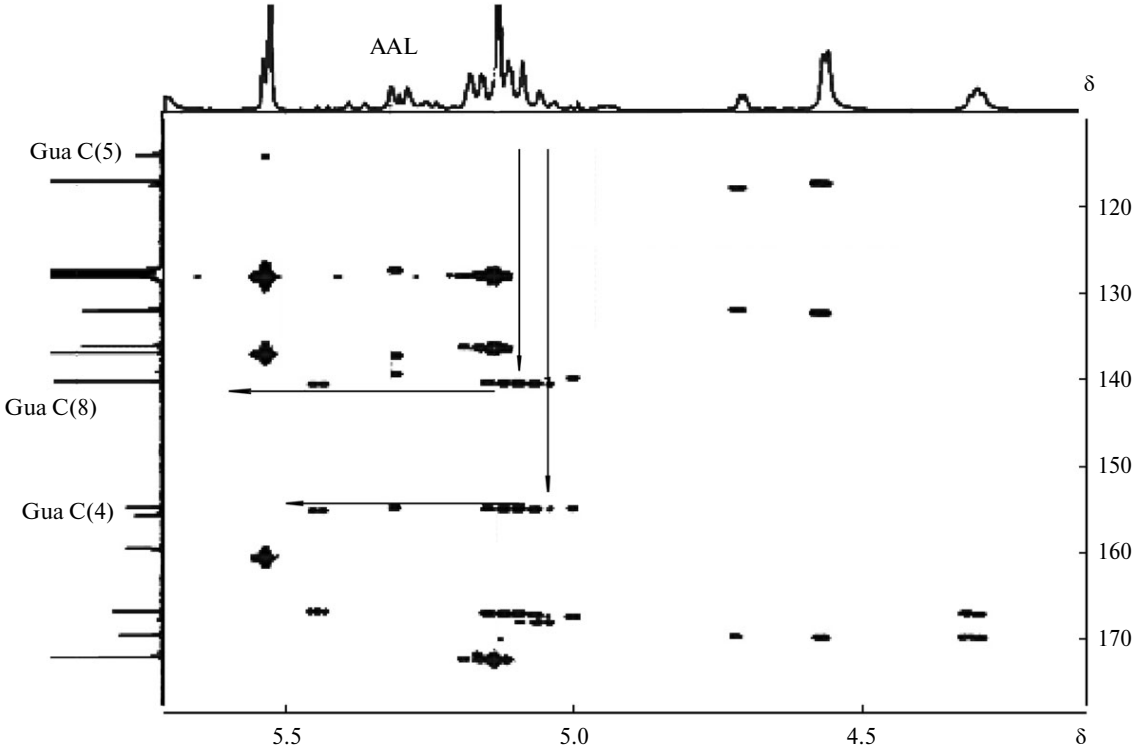


Fig. 2. A fragment of the HMBC spectrum of α-guanine-containing monomer **8**.

Earlier, we used to remove an allyl protection in the presence of morpholine and $Pd(PPh_3)_4$ as a catalyst, which gave moderate yields of the target products (45—53%).[25] An alternative procedure which used polymethylhydrosiloxane (PMHS)/$ZnCl_2$ (see Ref. 26) and a palladium catalyst did not increase the yields (<40%) of the target products. The $Pd(PPh_3)_4$-catalyzed removal of the *C*-terminal allyl protecting group in compounds **7** and **9** in the presence of *N*-ethylaniline[27] had proved to be the optimal and gave monomers **8** and **10** in 77 and 87% yields, respectively. The structure and purity of the synthesized compounds were confirmed by ^1H and ^{13}C NMR spectroscopy (no signals for the H and C atoms of the allyl group were observed in the spectra), elemental composition by the high resolution mass spectrometry data.

It is known that guanine derivatives are widely used as antineoplastic agents in the therapy of cancer diseases. For example, 6-*O*-benzylguanine **3** inhibits activity of alkyltransferase, thus decreasing toxicity of antitumor agents,[28] whereas 2-*N*-substituted purin analogs decrease the activity of proteinekinases.[29] In order to study biological activity of guanine derivatives **2** and **8**, we examined their inhibiting activity against enzyme topoisomerase I (Topo I). 6-*O*-Benzylguanine **3** was used as a comparison sample, which is known to inhibit this enzyme[3] (Fig. 3, *a*).

Topo I makes a supercoiled DNA (scDNA) to relax, resulting in the change of its conformation, which reflects

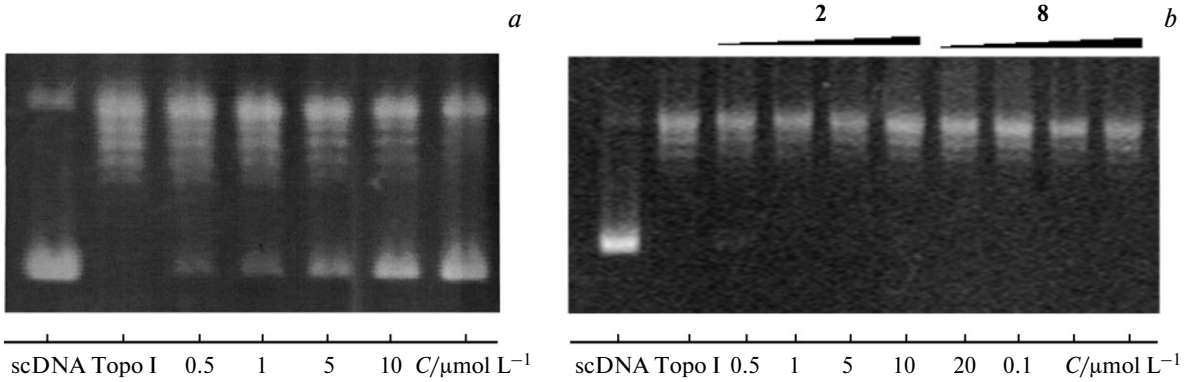


Fig. 3. Electrophoretograms of the relaxation reaction products of supercoiled DNA (scDNA) upon treatment with topoisomerase I (Topo I) in the presence of compound **3** (*a*) and compounds **2** and **8** (*b*).

in the change of the electrophoretic lability of its forms (topoisomers). The presence of the inhibitor in the studied range of concentrations hinders the scDNA relaxation, which is reflected in the more rapid migration in the gel as compared to the DNA relaxed with the tested enzyme.

Electrophoresis of the products of the reaction of scDNA with Topo I in the presence/absence of compounds **2** and **8** (see Fig. 3, *b*) showed that, in contrast to heterocyclic base **3** (see Fig. 3, *a*), these compounds do not inhibit (in the studied range of concentrations) the tested enzyme. Apparently, this can be due to the fact that the open positions N(2) and N(9) in guanine are necessary for the stronger binding with DNA, that hinders the Topo I activity.

In conclusion, the α-PNA guanine monomers **8** and **10** based on L-Glu were obtained by regioselective alkylation. It was also found that the order of addition of reagents is important in the acylation of the pseudopeptide with bromoacetyl bromide. It was shown that the diprotected guanine derivative **2** and the monoprotected monomer **8** did not inhibit Topo I activity. The use of diprotected guanine-containing monomer **10** seems to be the optimal for further oligomerization on a polymeric support, since it will be possible to carry out the capping step under the standard conditions (Ac_2O/TEA) and a qualitative Kaiser test in the course of the solid-phase synthesis of oligomers.

Experimental

The following commercially available reactants were used in the work: $[Pd(PPh_3)_4]$ (Sigma—Aldrich, USA), isobutyl chloroformate (Sigma—Aldrich, USA), 2-amino-6-chloropurin (Acros, Belgium), *N*-ethylaniline (Sigma—Aldrich, USA), bromoacetyl bromide (Sigma—Aldrich, USA); benzyl alcohol, acetic acid, triethylamine, THF, DMF, methanol, ethanol, dichloromethane, ethyl acetate, hexane, diethyl ether, P_2O_5, KOH, NaOH, $LiAlH_4$, sodium chloride, sodium sulfate, sodium hydride, calcium hydride, barium oxide, potassium carbonate — all of reagent or analytical grade (Russia). The following solvents were purified before use: benzyl alcohol (distilled *in high vacuo*), dichloromethane (distilled over P_2O_5), DMF (distilled over phthalic anhydride *in vacuo*), THF (distilled over KOH and directly before reactions over $LiAlH_4$), triethylamine (distilled over KOH and CaH_2).

NMR spectra were recorded on Bruker DPX-300 and Bruker DPX-600 pulse Fourier-transform spectrometers (Germany) at 25 °C relative to the internal standard Me_4Si.

High resolution mass spectra were recorded on a micrOTOF-Q II instrument (Bruker Daltonics GmbH, Germany). Values of optical angular rotation were determined on an AA-55 polarimeter (Optical Activity Ltd, Great Britain). Melting points were measured using a Carl Zeiss microscope.

Column chromatography was carried out on Silica gel 60 (0.040—0.063 mm) (Merck, Germany). Reaction progress was monitored by TLC on Silica gel 60 F_{254} plates (Merck, Germany). Compounds on the plates were visualized either under UV light (254 nm) or by spraying with a 0.5% solution of ninhydrin in ethanol or a mixture of molybdic acid—cerium(IV) sulfate monohydrate—concentrated sulfuric acid—water (2.5 : 1 : 3 : 94) with subsequent heating.

Solvents were evaporated on a vacuum rotary evaporator (14 Torr). The drying of compounds was finalized using a high vacuum of an oil pump (0.5 Torr).

6-*O*-Benzylguanine (3). Sodium hydride (1.2 g, 35.4 mmol, 55% suspension in mineral oil) was added to benzyl alcohol (50 mL) with vigorous stirring under argon. Then, 2-amino-6-chloropurin (2.0 g, 11.8 mmol) was added to the suspension obtained. The reaction mixture was heated to 60 °C and stirred for 12 h. After cooling, glacial acetic acid (1.4 mL, 24.3 mmol) was added to the suspension. The resulting mixture was extracted with 2 *M* solution of sodium hydroxide (5×5 mL). The organic phase was diluted with diethyl ether (100 mL) and again extracted with 2 *M* solution of sodium hydroxide (10×10 mL). The aqueous layers were combined, washed with diethyl ether (50 mL), glacial acetic acid was added to neutralize the solution (pH 7). Then, the solution was allowed to stand for 12 h at 4 °C. A precipitate formed was filtered and washed with water. After recrystallization (ethanol—water (1 : 1)), the crystals were dried *in vacuo*. The yield was 1.76 g (62%), yellow crystals, m.p. 201—202 °C. ^{1}H NMR (DMSO-d_6, 300 MHz), δ: 12.25 (br.s, 1 H, N(9)HGua); 7.82 (s, 1 H, C(8)HGua); 7.56—7.21 (m, 5 H, Ph); 6.29 (s, 2 H, NH_2Gua); 5.48 (s, 2 H, CH_2Ph(Bn)). ^{13}C NMR (DMSO-d_6, 75 MHz), δ: 159.7, 159.6, 156.1, 138.5, 136.8, 128.1, 126.5, 112.8, 66.7. Found (%): C, 56.0; H, 4.8; N, 27.5. $C_{12}H_{11}N_5O \cdot H_2O$. Calculated (%): C, 55.6; H, 5.1; N, 27.0. MS (ESI), found: *m/z* 243.1033 $[M + H]^+$. $C_{12}H_{12}N_5O$. Calculated: *M* = 242.1042.

6-*O*-Benzyl-2-*N*-(benzyloxycarbonyl)guanine (2). A suspension of NaH (1.74 g, 50.0 mmol, 55% in mineral oil) was washed with hexane, suspended in anhydrous DMF (50 mL), cooled to 0 °C, followed by an in portions addition of compound **3** (3.0 g, 12.45 mmol). The suspension was stirred for 15 min, followed by a dropwise addition of CbzCl (3.5 mL, 24.9 mmol). The mixture obtained was stirred for 12 h and neutralized by the addition of glacial acetic acid (pH 7), DMF was evaporated on a rotary evaporator. The residue was dissolved in CH_2Cl_2 (50 mL) and washed with water (2×20 mL). The organic layer was dried with Na_2SO_4, the solvent was evaporated. The product was isolated by column chromatography (eluent methanol—CH_2Cl_2 gradient, 0—10%) and dried *in vacuo*. The yield was 2.05 g (44%), white crystals, R_f 0.56 (CH_2Cl_2—methanol (8 : 1)), m.p. 207—209 °C. ^{1}H NMR (DMSO-d_6, 300 MHz), δ: 10.33 (s, 1 H, CbzNH—); 8.18 (s, 1 H, C(8)HGua); 7.77—7.03 (m, 10 H, 2 Ph(Cbz, Bn); 5.58 (s, 2 H, CH_2Ph(Cbz)); 5.18 (s, 2 H, CH_2Ph(Bn)). ^{13}C NMR (DMSO-d_6, 75 MHz), δ: 159.3, 156.2, 152.5, 152.1, 142.4, 137.1, 136.8, 129.4, 128.9, 128.7, 128.4, 128.3, 115.8, 67.9, 66.1. Found (%): C, 63.58; H, 4.77; N, 18.55. $C_{20}H_{17}N_5O_3$. Calculated (%): C, 63.99; H, 4.56; N, 18.66. MS (ESI), found: *m/z* 376.1388 $[M + H]^+$. $C_{20}H_{18}N_5O_3$. Calculated: *M* = 376.1410.

α-Allyl γ-benzyl *N*′-bromoacetyl-*N*-[2-(*tert*-butyloxycarbonyl)aminoethyl]-L-glutamate (5). Bromoacetyl bromide (1.30 mL, 14.60 mmol) was added to a solution of compound **4** (5.11 g, 12.17 mmol) in CH_2Cl_2 (50 mL) at 0 °C, followed by a dropwise addition of Et_3N (2.54 mL, 18.25 mmol) at such a rate that to keep the temperature of the mixture below 10 °C, then the mixture was stirred for 1 h. The solvent was evaporated, the residue was dissolved in ethyl acetate (50 mL) and sequentially washed

with water (2×40 mL) and saturated aqueous NaCl (2×30 mL). The organic phase was dried with Na_2SO_4, the solvent was evaporated. The product was isolated by column chromatography (eluent hexane—ethyl acetate (2 : 3)) and dried *in vacuo*. The yield was 4.70 g (85%), an yellow oil, R_f 0.65 (hexane—ethyl acetate (1 : 1)), $[\alpha]_D^{20}$ −10.0 (*c* 1.0, MeOH). ^{1}H NMR ($CDCl_3$, 300 MHz), δ: 7.35—7.28 (m, 5 H, Ph(Bn)); 5.91—5.85 (m, 1 H, CH_2—CH=CH_2); 5.36—5.24 (m, 2 H, —CH_2—CH=CH_2); 5.12, 5.05 (both d, 1 H each, CH_2Ph(Bn), *J* = 7.5 Hz); 4.62—4.48 (m, 2 H, CH_2—CH=CH_2); 3.91—3.82 (m, 2 H, N(C=O)CH_2—(AAL)); 3.75—3.71 (d, 1 H, CH(Gluα), *J* = 10.8 Hz); 3.62—3.43 (m, 1 H, BocHNCH_2CH_2); 3.32—3.21 (m, 1 H, BocHNCH_2CH_2); 3.07—2.94 (m, 2 H, BocHNC$H_2CH_2CH_2$); 2.55—2.25 (m, 4 H, CH_2(Gluγ), CH_2(Gluβ)); 1.39 (s, 9 H, Bu^t). ^{13}C NMR ($CDCl_3$, 75 MHz), δ: 172.1, 169.5, 167.0, 155.2, 134.9, 130.6, 127.8, 127.6, 127.4, 118.5, 79.0, 65.9, 65.7, 65.4, 58.6, 49.2, 37.7, 29.2, 27.9, 25.5, 22.7. MS (ESI), found: *m/z* 563.1390 $[M + Na]^+$. $C_{24}H_{33}N_2O_7BrNa$. Calculated: *M* = 563.1369.

α-Allyl γ-benzyl *N*-[(6-*O*-benzyl)guanin-9-ylacetyl]-*N*-[2-(*tert*-butyloxycarbonyl)aminoethyl]-L-glutamate (7). A predried potassium carbonate (232 mg, 1.682 mmol) was added to a suspension of compound **3** (406 mg, 1.682 mmol) in freshly distilled DMF (5 mL) with mechanical stirring. The reaction mixture was stirred for 15 min at ~20 °C, followed by a dropwise addition of a solution of compound **5** (455 mg, 0.841 mmol) in DMF (5 mL). After 12 h, the solvent was evaporated, the mixture obtained was dissolved in ethyl acetate (50 mL), filtered through a layer of silica gel (10×10 mm), and washed with water (2×20 mL) and saturated aqueous NaCl (2×20 mL). The organic phase was dried with Na_2SO_4, the solvent was evaporated on a rotary evaporator. The product was subjected to chromatography, using ethyl acetate as the eluent. The solvent was evaporated, the compound was dried *in vacuo*. The yield was 360 mg (61%), white crystals, R_f 0.63 (ethyl acetate), m.p. 51—53 °C, $[\alpha]_D^{20}$ −1.7 (*c* 1.0, MeOH). ^{1}H NMR (acetone-d_6, 600 MHz), δ: 7.69 (s, 1 H, C(8)HGua); 7.58—7.24 (m, 10 H, 2 Ph(Bn)); 6.23 (s, 1 H, BocNH); 5.95—5.84 (m, 1 H, CH_2—CH=CH_2); 5.74 (s, 2 H, NH_2Gua), 5.56—5.52 (d, 2 H, CH_2Ph(Bn)Gua, *J* = 7.2 Hz); 5.34, 5.17 (both d, 1 H each, CH_2—CH=CH_2, *J* = 17.2 Hz); 5.15 (d, 2 H, CH_2Ph(Bn), *J* = 3.2 Hz); 5.13 (d, 2 H, —N(C=O)CH_2—(AAL), *J* = 14.8 Hz); 4.59—4.55 (m, 2 H, CH_2—CH=CH_2); 4.35—4.25 (t, 1 H, CH(Gluα), *J* = 5.9 Hz); 3.91—3.43 (m, 4 H, BocNHCH_2CH_2—); 2.59—2.54 (t, 2 H, CH_2(Gluγ), *J* = 7.4 Hz); 2.45—2.37 (m, 2 H, CH_2(Gluβ)); 1.37 (s, 9 H, Bu^t). ^{13}C NMR (acetone-d_6, 150 MHz), δ: 174.0 (C=O(GluγOBn)); 171.5 (C=O(COOAll); 168.7 (N(C=O)-CH_2—(AAL)); 162.3 (CH_2Ph(Bn)Gua); 161.4 (C(2)HGua); 157.6 (C=O(Boc)); 156.6 (C(4)Gua); 142.1 (C(8)HGua); 138.8 (C(Ph)); 134.0 (CH_2—CH=CH_2); 130.2—129.4 (CH(Ph)); 119.0 (CH_2—CH=CH_2); 115.9 (C(5)Gua); 80.1 (C—O(Boc)); 68.8 (CH_2(Bn)Gua); 67.4 (CH_2(Bn)); 66.9 (CH_2—CH=CH_2); 61.5 (CH_2(Gluα)); 49.7 (BocNHCH_2); 45.1 (N(C=O)CH_2—(AAL)); 40.8 (BocNHCH_2CH_2); 31.9 (CH_2(Gluγ)); 29.3 (CH_3(Boc)); 25.8 (CH_2(Gluβ)). MS (ESI), found: *m/z* 702.3231 $[M + H]^+$. $C_{36}H_{44}N_7O_8$. Calculated: *M* = 702.3251.

α-Allyl γ-benzyl *N*-[(2-*N*-benzyloxycarbonyl-6-*O*-benzyl)-guanin-9-ylacetyl]-*N*-[2-(*tert*-butyloxycarbonyl)aminoethyl]-L-glutamate (9). A predried potassium carbonate (111 mg, 0.8 mmol) was added to a suspension of compound **2** (300 mg, 0.8 mmol) in freshly distilled DMF (5 mL) with mechanical stirring. The reaction mixture was stirred for 15 min at ~20 °C, followed by a dropwise addition of a solution of compound **5** (333 mg, 0.62 mmol) in DMF (5 mL). After 12 h, the solvent was evaporated, the mixture obtained was dissolved in ethyl acetate (50 mL), filtered through a layer of silica gel (10×10 mm), and washed with water (2×20 mL) and a saturated aqueous NaCl (2×20 mL). The organic phase was dried with Na_2SO_4, the solvent was evaporated. The product was subjected to chromatography, using ethyl acetate as the eluent. The solvent was evaporated, the compound was dried *in vacuo*. The yield was 200 mg (41%), white crystals. R_f 0.71 (ethyl acetate), $[\alpha]_D^{20}$ −1.7 (*c* 1.0, MeOH), m.p. 47—49 °C. ^{1}H NMR ($CDCl_3$, 300 MHz), δ: 7.70 (s, 1 H, C(8)HGua); 7.50—7.30 (m, 15 H, 3 Ph(Cbz, Bn)); 5.82—5.75 (m, 1 H, CH_2CH=CH_2); 5.61 (s, 1 H, BocNH); 5.40—4.29 (m, 12 H, CH_2CH=CH_2, 3 CH_2Ph, N(C=O)CH_2—(AAL), CH_2CH=CH_2); 3.99—3.82 (m, 1 H, CH(Gluα)); 3.70—3.62 (m, 1 H, BocHNCH_2CH_2); 3.45—3.33 (m, 1 H, BocHNCH_2CH_2); 3.30—3.18 (m, 2 H, BocNHCH_2CH_2); 2.50—2.30 (m, 4 H, CH_2(Gluγ), CH_2(Gluβ)); 1.39 (s, 9 H, Bu^t). ^{13}C NMR ($CDCl_3$, 75 MHz), δ: 173.0, 170.2, 167.0, 161.1, 159.3, 156.0, 154.1, 140.0, 136.5, 135.4, 131.4, 128.6, 119.3, 119.1, 80.1, 68.1, 66.9, 66.7, 66.3, 60.09, 48.9, 44.2, 38.8, 30.3, 28.7, 24.0. MS (ESI), found: *m/z* 858.3411 $[M + Na]^+$. $C_{44}H_{49}N_7O_{10}Na$. Calculated: *M* = 858.3439.

γ-Benzyl *N*-[(6-*O*-benzyl)guanin-9-ylacetyl]-*N*-[2-(*tert*-butyloxycarbonyl)aminoethyl]-L-glutamate (8). *N*-Ethylaniline (0.32 mL, 2.06 mmol) and $Pd(PPh_3)_4$ (59 mg, 0.05 mmol) were sequentially added to a suspension of compound **7** (360 mg, 0.51 mmol) in THF (4 mL) under argon. After 1 h, the reaction mixture was added dropwise to hexane (100 mL), this resulted in the formation of a precipitate, which was filtered, washed with hexane, and subjected to chromatography, using ethyl acetate as the eluent. The solvent was evaporated, the compound was dried *in vacuo*. The yield was 260 mg (77%), yellow crystals. R_f 0.50 (ethyl acetate), m.p. 120—122 °C, $[\alpha]_D^{20}$ −1.7 (*c* 1.0, MeOH). ^{1}H NMR (DMSO-d_6, 300 MHz), δ: 7.72 (d, 1 H, *J* = 6.5 Hz); 7.55—7.25 (m, 10 H); 7.13—6.95 (m, 1 H); 6.39 (s, 2 H); 5.50 (s, 2 H); 5.16—4.89 (m, 4 H); 4.31—4.00 (m, 1 H); 3.52—2.96 (m, 4 H); 2.69—2.53 (m, 1 H); 2.42—2.19 (m, 2 H), 2.04—1.86 (m, 1 H), 1.41 (s, 9 H). ^{13}C NMR (DMSO-d_6, 75 MHz), δ: 172.8, 172.6, 167.7, 166.9, 160.1, 159.8, 155.7, 141.1, 137.0, 136.3, 131.7, 128.9, 128.8, 128.4, 128.1, 121.3, 113.3, 78.2, 77.6, 67.0, 65.6, 61.1, 59.8, 46.2, 44.6, 39.7, 31.4, 28.4, 25.0. MS (ESI), found: *m/z* 660.2790 $[M - H]^-$. $C_{33}H_{38}N_7O_8$. Calculated: *M* = 660.2782.

γ-Benzyl *N*-[(2-*N*-benzyloxycarbonyl-6-*O*-benzyl)guanin-9-ylacetyl]-*N*-[2-(*tert*-butyloxycarbonyl)aminoethyl]-L-glutamate (10). *N*-Ethylaniline (0.12 mL, 0.78 mmol) and $Pd(PPh_3)_4$ (23 mg, 0.02 mmol) were sequentially added to a suspension of compound **9** (164 mg, 0.20 mmol) in THF (4 mL) under argon. After 1 h, the reaction mixture was added dropwise to hexane (100 mL), this resulted in the formation of a precipitate, which was filtered, washed with hexane, and subjected to chromatography, using ethyl acetate as the eluent. The solvent was evaporated, the compound was dried *in vacuo*. The yield was 154 mg (87%), yellow crystals, R_f 0.61 (CH_2Cl_2—MeOH—AcOH (9 : 1 : 0.1)), m.p. 116—118 °C, $[\alpha]_D^{20}$ −5.0 (*c* 1.0, CH_3OH). ^{1}H NMR (DMSO-d_6, 300 MHz), δ: 10.35 (d, 1 H, *J* = 7.1 Hz); 7.72 (d, 1 H, *J* = 5.2 Hz); 7.65—7.12 (m, 15 H); 6.98—6.73 (m, 1 H); 5.59 (s, 2 H); 5.29—4.89 (m, 6 H); 4.31—3.99 (m, 1 H); 3.21—2.83 (m, 3 H); 2.71—2.51 (m, 1 H); 2.43—2.15 (m, 2 H); 2.04—1.80 (m, 2 H); 1.35 (s, 9 H). ^{13}C NMR (DMSO-d_6,

75 MHz), δ: 173.4, 173.2, 160.2, 160.0, 156.0, 152.3, 137.1, 136.6, 134.9, 131.9, 129.3, 128.7, 128.3, 128.2, 116.9, 110.0, 78.4, 78.0, 68.1, 66.1, 65.9, 65.8, 45.0, 38.7, 31.7, 28.9, 25.5. MS (ESI), found: m/z 794.3155 $[M - H]^-$. $C_{41}H_{44}N_7O_{10}$. Calculated: $M = 794.3150$.

Biological trials. The ability of compounds **2**, **3**, and **8** to inhibit activity of Topo I *in vitro* was determined in the relaxation reaction of scDNA. The reaction mixture (20 μL) containing Topo I (1—2 activity units) (Promega, USA) was incubated with supercoiled plasmid DNA pBR_{322} (205 ng) (Fermentas, Lithuania) in the reaction buffer (35 m*M* Tris-HCl, pH 8.0, 72 m*M* KCl; 5 m*M* $MgCl_2$ containing 5 m*M* dithiothreitol; 2 m*M* spermidine, 100 mg mL^{-1} of bovine serum albumine) (all the reagent from Sigma, USA) in the presence of 0.1% DMSO (control of the solvent, the data are not shown) and compounds under study at 37 °C for 30 min. The reaction was stopped by the addition of sodium dodecylsulfate (to 1%). After addition of proteinase K (the final concentration 50 mg mL^{-1}), the reaction mixture was incubated for 30—40 min at 37 °C. DNA-topoisomers were separated using electrophoresis in 1% agarose body (3 h, 60 V) in a buffer containing 40 m*M* Tris-base, 1 m*M* EDTA, 30 m*M* glacial acetic acid, after the addition (1 : 10) of the buffer for the deposition (0.25% bromophenol blue, 50% glycerol). The gels after electrophoresis were colored with ethidium bromide and visualized under UV light.

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References

1. G. Ferenc, P. Pádár, J. Szolomájer, L. Kovács, *Curr. Org. Chem.*, 2009, **13**, 1085.
2. P. Pourquier, J. L. Waltman, Y. Urasaki, N. A. Loktionova, A. E. Pegg, J. L. Nitiss, Y. Pommier, *Cancer Res.*, 2001, **61**, 53.
3. L. H. Jensen, A. V. Thougaard, M. Grauslund, B. Søkilde, E. V. Carstensen, H. K. Dvinge, D. A. Scudiero, P. B. Jensen, R. H. Shoemaker, M. Sehested, *Cancer Res.*, 2005, **65**, 7470.
4. P. E. Nielsen, M. Egholm, R. H. Berg, O. Buchardt, *Science*, 1991, **254**, 1497.
5. P. E. Nielsen, *Chem. Biodiversity*, 2010, **7**, 786.
6. B. Hyrup, M. Egholm, P. E. Nielsen, P. Wittung, B. Norde´n, O. Buchardt, *J. Am. Chem. Soc.*, 1994, **116**, 7964.
7. P. Wittung, P. E. Nielsen, O. Buchardt, M. Egholm, B. Norde´n, *Nature*, 1994, **368**, 561.
8. V. Demidov, V. N. Potaman, M. D. Frank-Kamenetskii, O. Buchardt, M. Egholm, P. E. Nielsen, *Biochem. Pharmacol.*, 1994, **48**, 1309.
9. V. A. Efimov, O. G. Chakhmakhcheva, E. Wickstrom, *Nucleosides, Nucleotides and Nucleic Acids*, 2005, **24**, 1853.
10. V. A. Efimov, K. R. Birikh, D. B. Staroverov, S. A. Lukyanov, M. B. Tereshina, A. G. Zaraisky, O. G. Chakhmakhcheva, *Nucl. Acids Res.*, 2006, **34**, 2247.
11. R. Corradini, S. Sforza, T. Tedeschi, F. Totsingan, A. Manicardi, R. Marchelli, *Curr. Top. Med. Chem.*, 2011, **11**, 1535.
12. K. Usui, A. Okada, K. Kobayashi, N. Sugimoto, *Org. Biomol. Chem.*, 2015, **13**, 2022.
13. L. Christensen, R. Fitzpatrick, B. Gildea, K. H. Petersen, H. F. Hansen, T. Koch, M. Egholm, O. Buchardt, P. E. Nielsen, J. Coull, R. H. Berg, *J. Pept. Sci.*, 1995, **3**, 175.
14. A. Porcheddu, G. Giacomelli, I. Piredda, M. Carta, G. Nieddu, *Eur. J. Org. Chem.*, 2008, 5786.
15. S. Pothukanuri, Z. Pianowski, N. Winssinger, *Eur. J. Org. Chem.*, 2008, 3141.
16. K. L. Dueholm, M. Egholm, C. Behrens, L. Christensen, H. F. Hansen, T. Vulpius, K. H. Petersen, R. H. Berg, P. E. Nielsen, O. Buchardt, *J. Org. Chem.*, 1994, **59**, 5767.
17. P. C. Meltzer, A. Y. Liang, P. Matsudaira, *J. Org. Chem.*, 1994, **60**, 4305.
18. A. V. Dezhenkov, M. V. Tankevich, E. D. Nikolskaya, I. P. Smirnov, G. E. Pozmogova, V. I. Shvets, Yu. G. Kirillova, *Mendeleev Commun.*, 2015, **25**, 47.
19. S. A. Thomson, J. A. Josey, R. Cadilla, M. D. Gaul, C. F. Hassman, M. J. Luzzio, A. J. Pipe, K. L. Reed, D. J. Ricca, R. W. Wiethe, S. A. Noble, *Tetrahedron*, 1995, **51**, 6179.
20. Z. Timár, L. Kovács, G. Kovács, Z. Schmél, *J. Chem. Soc., Perkin Trans.*, 2000, **1**, 19.
21. Haaima, A. Lohse, O. Buchardt, P. E. Nielsen, *Angew. Chem., Int. Ed.*, 1996, **98**, 1939.
22. C. Barth, O. Seitz, H. Kunz, *Z. Naturforsch.*, 2004, **59b**, 802.
23. N. P. Boyarskaya, D. I. Prokhorov, Y. G. Kirillova, E. N. Zvonkova, V. I. Shvets, *Tetrahedron Lett.*, 2005, **46**, 7359.
24. A. V. Baranov, N. S. Tsvid, V. I. Luk´yanchenko, D. I. Prokhorov, Yu. G. Kirillova, V. I. Shvets, *Vestn. MITKhT* [*Bull. Moscow Inst. Fine Chem. Technol.*], 2007, **2**, No. 5, 28 (in Russian).
25. S. Friedrich-Bochnitschek, H. Waldmann, H. Kunz, *J. Org. Chem.*, 1989, **54**, 751.
26. S. Chandrasekhar, Ch. R. Reddy, R. J. Rao, *Tetrahedron*, 2001, **57**, 3435.
27. T. Shiraishi, R. Hamzavi, P. E. Nielsen, *Nucl. Acids Res.*, 2008, **36**, 4424.
28. M. E. Dolan, A. E. Pegg, *Clinical Cancer Res.*, 1997, **3**, 837.
29. N. S. Gray, L. Wodicka, A.-M. W. H. Thunnissen, T. C. Norman, S. Kwon, F. H. Espinoza, D. O. Morgan, G. Barnes, S. LeClerc, L. Meijer, S.-H. Kim, D. J. Lockhart, P. G. Schultz, *Science*, 1998, **281**, 533.

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