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Potentiation of BCNU Cytotoxicity by Molecules Targeting Abasic Lesions in DNA

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Abstract—We describe the synthesis, DNA binding measurements and pharmacological properties of a series of new heterodimeric molecules, in which a 2,6-diaminopurine is linked to a 9-aminoacridine chromophore. The linking chain contains a central *N*,*N*′-disubstituted guanidine, connected to the two chromophores by polymethylenic units of variable length. © 2001 Elsevier Science Ltd. All rights reserved.

The pharmacological properties of many antitumor drugs are due to their ability to alter DNA. In particular, alkylating agents that covalently bind to the nucleic bases, induce major changes in the macromolecule.[1] Most of these lesions are recognized and repaired by specific enzymes. This repair activity is critical for cells as the persistence of such lesions could be mutagenic and even lethal. The therapeutic effect of high doses of antitumor drugs is mainly due to the production of multiple lesions and to the saturation of the repair machinery.[2] Therefore, the design of drugs able to interfere with DNA repair constitutes an attractive strategy to potentiate the action of known anticancer agents, allowing the use of lower doses to achieve comparable therapeutic result.[3] A number of enzymes are involved in DNA repair. The subject has been recently reviewed.[4] The major repair process of alkylated nucleic bases (Base excision repair or BER) begins with the excision of the modified bases by specific glycosylases, thus creating abasic sites.[5] Abasic sites or AP-sites (AP for apurinic or apyrimidinic),[6] which also form spontaneously or under the action of physical agents such as UV or γ-radiation, are recognized by AP-endonucleases that are specialized enzymes able to cleave DNA strands at AP-sites. Their action constitutes the first stage of the repair of such lesions by a cascade of specific enzymes. By their strategic position in the repair process and their biological importance, AP-sites appear to be attractive target candidates for potentiation of alkylating antitumor agents.[7] Simple molecules such as methoxyamine,[8] 9-aminoellipticine[9] or isopropyloxazolocarbazole (Ipr-OPC)[10] have been proposed as AP-endonuclease inhibitors. Their hypothetical mechanism of action is based on covalent binding to the aldehydic form of the abasic site.

We have previously reported molecules that specifically interact at AP-sites through another process.[11–14] These molecules are constituted of three units: (1) an intercalator for targeting DNA, (2) a nucleic acid base for the recognition of the abasic site, and (3) a polyamino linker able to stabilize the drug/DNA complex by electrostatic interactions with the phosphate backbone. The biochemical and biophysical properties of these heterodimers are strongly dependent on the nature of the linker. Molecules **1** and **2** that contain secondary amines display strong DNA cleavage activity at abasic sites and act as artificial endonucleases[11] while compound **3**, that contains two guanidines in the linker, shows a strong affinity for abasic site containing duplexes but does not cleave DNA.[14] In molecules **1** and **2**, the amines of the linker play a dual action, one amino group being essentially protonated ($pK_a = 8.5$ and 8.1 for **1** and **2** respectively) increases affinity for DNA, while the second amine being essentially unprotonated ($pK_a = 6.5$ and 6.7 for **1** and **2**) cleaves the phosphodiester backbone following a β elimination mechanism.[15] The guanidine residues of **3** participate only to the stabilization of the drug/DNA complex. Compound **3** was shown to potentiate, both in vitro and in vivo, the activity of the alkylating antitumor agent, bis(chloroethyl)nitrosourea (BCNU),[14] while molecules **1** and **2** exhibit only little

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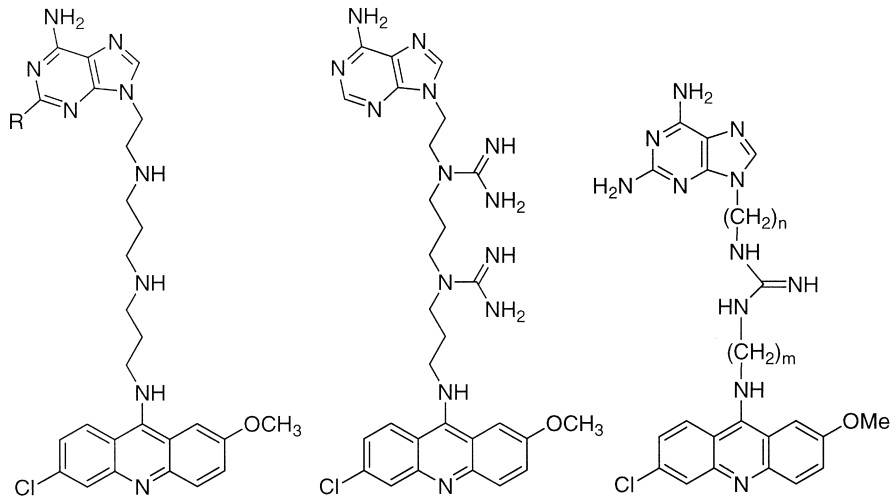


1 : R = H
2 : R = NH_2

3

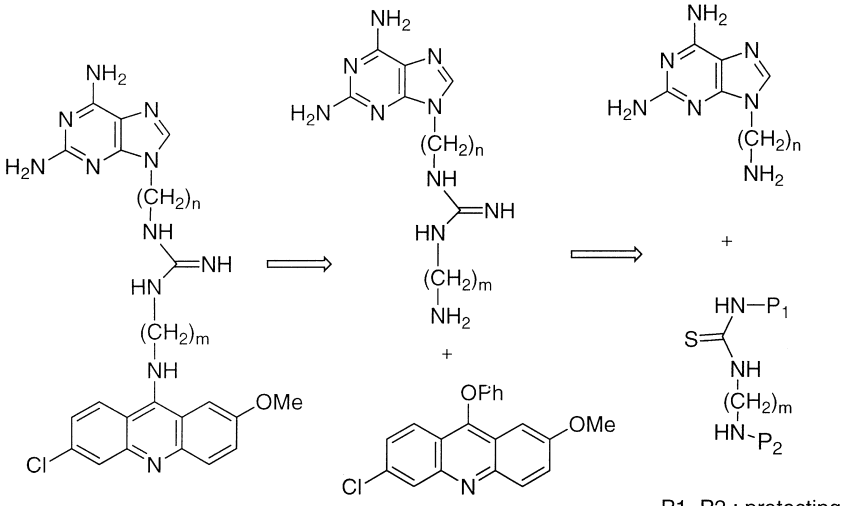
synergistic action in certain tests.[7,14] These results suggest that targeting the abasic site might be a realistic strategy to potentiate the action of alkylating drugs.

One limitation with compound **3** is a curare-like acute toxicity in vivo, which was tentatively attributed to the two guanidine groups. With the aim of modulating both pharmacological activity and toxicity, and further to ascertain this abasic site targeting strategy, we designed new compounds containing a single guanidine in the linker. In this new series, all molecules contain the 9-amino-6-chloro-2-methoxyacridine intercalating chromophore, and 2,6-diaminopurine was preferred to adenine as abasic site recognition unit, as it was shown in previous studies to confer higher binding affinity for DNA to the heterodimers.[11] The length of the polymethylene units connecting the guanidine to the two heterocycles were varied in this study, and their influence on DNA binding and pharmacological properties was evaluated.

Results and Discussion

Synthesis

As shown in the retrosynthetic pathway depicted in Figure 1, the molecules were prepared by step by step construction of the guanidine linker. The acridine moiety was introduced in the last step. Various procedures are reported in the literature to prepare *N*,*N*′-disubstituted guanidines.[16–24] Different methods were tested and the best result was achieved using the method



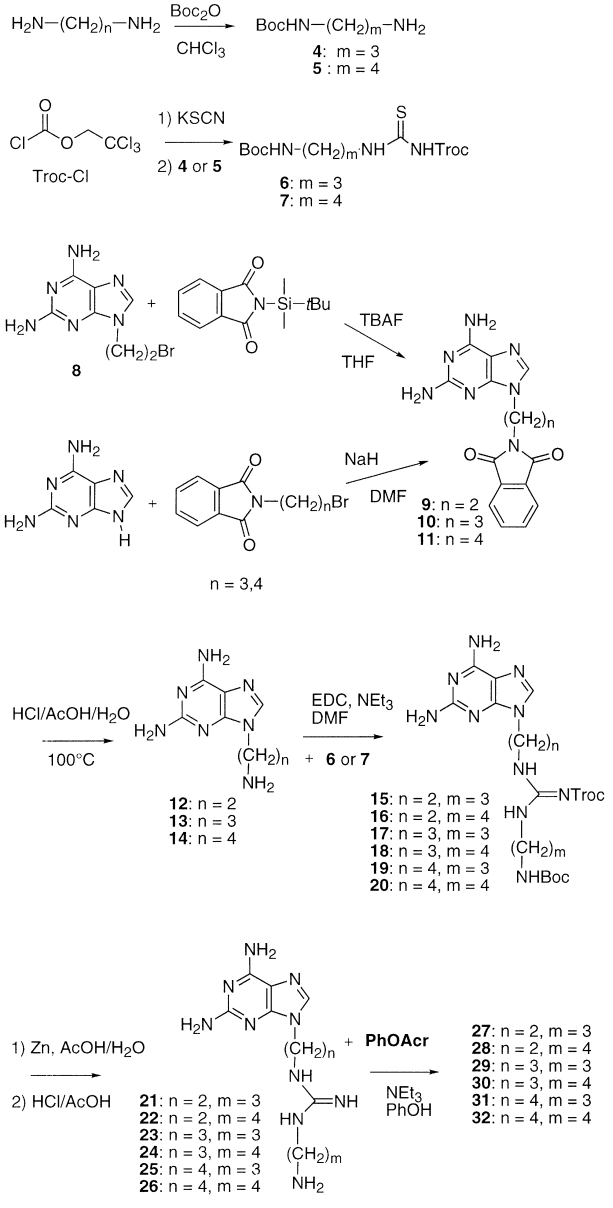
27: n = 2, m = 3
28: n = 2, m = 4
29: n = 3, m = 3
30: n = 3, m = 4
31: n = 4, m = 3
32: n = 4, m = 4

PhOAcr

P1, P2 : protecting groups

Figure 1. Retrosynthetic strategy.

reported by Poss,[23] in which a *N*-protected thiourea was reacted with a primary amine in the presence of the coupling reagent EDC (*N*-diethylaminopropyl-*N*-ethyl-carbodiimide, hydrochloride). As described in Scheme 1 the key thiourea intermediates **6** and **7** were prepared one-pot by reaction of potassium thiocyanate with trichloroethyl-chloroformate,[16] followed by addition of the Boc-protected diaminoalkane (**4** or **5**). The Boc and Troc protected thiourea synthons **6** and **7** were thus obtained in good yields (83 and 86%, respectively). Two methods were used to prepare the 9-aminoalkyl-2,6-diaminopurines [DAP–$(CH_2)_n$–NH_2, **12**–**14**]. The propyl and butyl analogues **13** and **14** were prepared following the method reported by Leonard for the alkylation of adenine,[25] that's by treating 2,6-diaminopurine (DAP) with the ω-bromo-alkylphthalimides in the presence of sodium hydride. The reaction took place regioselectively at position 9, no alkylation at N-7 was observed. Deprotection of the amine was achieved by hydrolysis of the phthalimido group in acidic conditions (HCl/$AcOH/H_2O$) affording compounds **13** and **14** in 72 and 74% yields, respectively. The aminoethyl analogue **12** was prepared in a different way. As observed previously for the analogue containing adenine instead of DAP,[26] the reaction with 2-bromoethylphthalimide gave very poor yield of 9-alkylated DAP **9**. Compound **9** was therefore prepared by reacting 9-(2-bromoethyl)-2,6-diaminopurine **8**[11] with *tert*-butyl-dimethylsilyl phthalimide[27] in the presence of fluoride ion. Deprotection of the amino group was achieved by acid hydrolysis (HCl/$AcOH/H_2O$) and gave the amine **12** in 72% yield. Reaction of the aminoalkyl-DAP **12**–**14** with the protected thioureas **6** or **7** in the presence of coupling agent EDC gave the Boc,Troc protected guanidines **15**–**20** as oily products (60–80% yields, purity >95%). If necessary, purification was achieved by column chromatography. Due to the high polarity of the products, yields in pure compounds dramatically drop to 12% after chromatography. Orthogonal deprotections of the two protecting groups were tested, but as it appeared that aminoacridines decomposed in the conditions required to cleave the Troc protecting groups (zinc in acetic acid), the two groups were cleaved prior to introduction of the acridine nucleus. One-pot deprotection was thus achieved by treatment of the protected guanidines **15**–**20** with a suspension of zinc in acetic acid, followed by acidic hydrolysis (1 N solution of hydrochloric acid in acetic acid). The purity of the resulting oils was checked by hplc (purity ≥95%) and compounds **21**–**26** were used without further purification. NMR analysis confirmed the complete removal of the two protecting groups. Final reaction of the primary aliphatic amines present in **21**–**26** with 6-chloro-2-methoxy-9-phenoxyacridine (PhOAcr) was performed in phenol as previously described.[11] Compounds **27**–**32** were isolated in 9–54% yields depending on the purification steps.



Scheme 1. Syntheses.

Interaction with calf-thymus DNA

The binding constants of the different molecules for calf-thymus DNA (CT-DNA) were measured by competition with ethidium bromide (EB). Studies on depurinated DNA are precluded because of inherent instability of the abasic sites that easily undergo strand scission. Data are collected in Table 1.

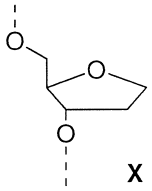
The molecules bind strongly to native DNA ($K_a = 2$–8×10^5 M^{-1}) with the exception of **29** that displays a lower binding constant ($K_a = 0.6\times10^5$ M^{-1}). The number of covered sites is two, in agreement with the rule of the exclusion site for mono-intercalators. Compounds **27** and **28** with two methylene units connecting the guanidine to the purine show the highest affinities.

Interaction with AP-site containing oligonucleotides

In preceding paper,[13] we showed that denaturation study (T_m experiments) was a method of choice to compare the relative affinity of the series of heterodimers for abasic site containing oligonucleotides. Studies were performed on synthetic oligonucleotides containing a chemically stable analogue of the abasic

CGCAC**X**CACGC
GCGTGTGTGCG
Abasic site containing duplex (TX)

CGCAC**A**CACGC
GCGTGTGTGCG
Parent duplex (TA)



Stable analog of the abasic site

Scheme 2. Sequences of the synthetic oligonucleotides used in the T_m experiments.

lesion, 3-hydroxy-2-(hydroxymethyl)-tetrahydrofuran (designated as X). We used the sequence previously studied in our group (molecular modeling and high field nmr),[12–14] and containing a thymine opposite the lesion in the complementary strand for possible pairing with the DAP moiety of the drug. The sequences of the TX duplex containing the stable abasic site, and the parent TA duplex in which X is replaced by adenine, are shown in Scheme 2. The thermal denaturation studies were monitored by adding increased concentrations of each drug to the duplexes. To compare the relative affinities of the drugs, we used ΔT_m values [$\Delta T_m = T_m$ (duplex in the presence of the drug)$-T_m$ (duplex alone)]. Results are given in Figure 2.

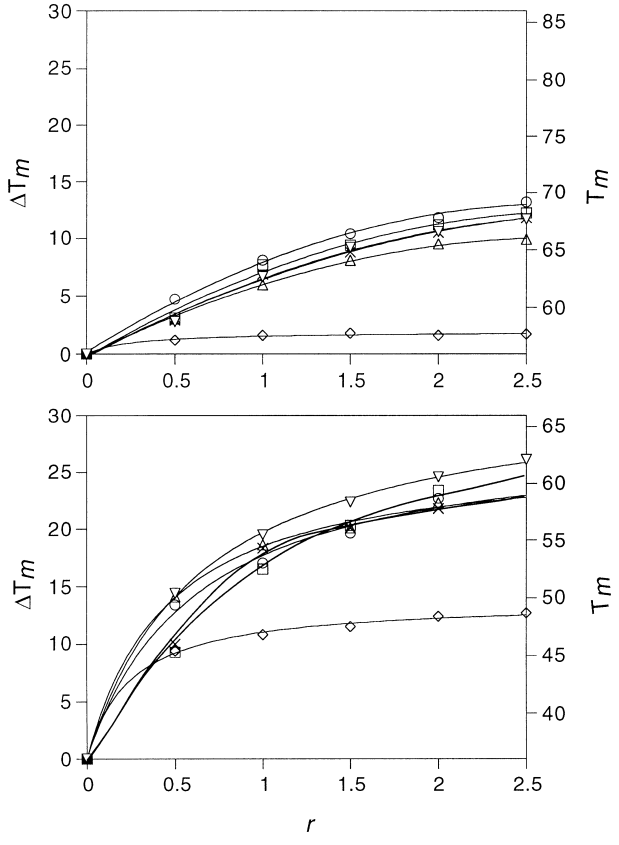


Figure 2. Influence of the drugs on the T_m and ΔT_m of the TA (above) and TX (below) duplexes. Experiments were conducted at pH 7 (10 mM sodium phosphate, 1 mM EDTA, 20 mM NaCl). The drugs tested were (○) **27**, (□) **28**, (◇) **29**, (X) **30**, (△) **31**, and (▽) **32**. ΔT_m is the difference between the T_m of the duplex in the presence of the drug and the T_m of the duplex alone. T_m values were measured for various ratios r of drugs (r = [drug]/[Duplex]).

If we first consider the results observed with the 'unmodified' TA duplex, the five compounds **27**, **28**, **30**, **31** and **32** stabilize the duplex as shown by the regular increase of the T_m values when the drug to DNA ratio (r) increases. The shapes and amplitudes of the curves are very similar to what was previously observed for compounds **1**–**3**.[13,14] These data are interpreted in terms of a non-specific interaction as expected with a drug containing an acridine intercalator. With the abasic site containing TX duplex however, the behavior of the same five compounds (**27**, **28**, **30**, **31**, **32**) is quite different. First, the increase in the T_m values on drug addition is much higher. Second, the slopes of the curves are steeper at low drug to DNA ratios ($r < 1$) than for higher ratios ($r > 1$). The shapes of the curves suggest preferred formation of 1:1 complexes between the drugs and the oligonucleotide. A non-specific interaction is observed at drug to DNA ratios $r > 1$. This behavior again is quite similar to what was observed for compounds **1**–**3** in terms of comparable shapes and amplitudes of the curves.[13,14] In these melting temperature experiments monitored both with the abasic and the parent duplexes the curves obtained for compound **29** differ considerably from those observed for the five analogues (**27**, **28**, **30**–**32**). Apparently, **29** does not stabilize the parent TA duplex, which correlates the lower affinity constant measured with CT-DNA, while it causes stabilization of the abasic TX duplex to an extent that culminates at a 1:1 ratio. These data suggest very specific interaction of the drug at the abasic site.[28]

Pharmacological data

In vitro pharmacological evaluation of the target compounds **27**–**32** was carried out as previously reported.[14] Intrinsic cytostatic activity was estimated by measuring the dose-dependent growth inhibition of L1210 murine leukemia cells. Cytostatic/cytotoxic properties as well as synergistic potency with BCNU were determined by measuring the clonogenicity of A549 human pulmonary carcinoma cells. Results are given in Table 1.

Cytostatic activity on L1210 cells appeared rather weak and cytotoxicity on A549 moderate; no apparent relationship was found between the length of the linker and toxicity. Compared to the parent compound **3**, cytotoxicities are within the same scale. Apparent synergy was measured by simultaneous exposition of the cells to 10 μM of the tested compound and 10 μM BCNU and expressed as the % of toxicity increase with regard to the addition of the toxic effects of both drugs alone taken as 100% (Fig. 3).

Three compounds, **29**, **30** and **32**, displayed significant synergy with BCNU. Interestingly, **29** and **32**, which are the most synergistic molecules, are among the weakest compounds in terms of toxicity on both cell lines.

Table 1. Binding constants and cytostatic/cytotoxic properties (alone and in association with BCNU) of compounds **27**–**32**: comparison with compounds **1**–**3** used as references

Compound (mn)	IC_{50} L1210 (μM) (growth inhibition)	IC_{50} A549 (μM) (clonogenicity inhibition)	Synergy[a] (%)	K_a ($\times 10^{-5}$ M^{-1})
27 (2,3)	74	3	—	6
28 (2,4)	26	6	—	8
29 (3,3)	>100	28	+74	0.6
30 (3,4)	60	7	+16	3.7
31 (4,3)	>100	31	—	2.1
32 (4,4)	>100	43	+49	4
3[b]	33	10	+94	20
1	>10[d]	0.1	+[c]	2
2	>10[d]	0.05	—	11

[a]Apparent synergy was measured by simultaneous exposition of A549 cells to 10 μM of the tested compound and 10 μM BCNU and expressed as the % of toxicity increase with regard to the addition of the toxic effects of both drugs alone (theoretical combined toxicity) taken as 100%.
[b]data from ref. 14.
[c]+25% synergy observed after exposition to 0.05 μM of 1 and 10 μM BCNU. Cytotoxicity was too strong at highest doses.
[d]IC_{50} of, respectively, 1.6 and 2.2 μM have been previously described after 48 h incubation with L1210 cells. Both compounds were shown synergistic with DNA damaging agents on this cell line.[7]

Although BCNU potentiation is slightly weaker with **29** than with **3**, its low cytotoxic properties make of **29** a good candidate for in vivo pharmacological and toxicological studies and compel further investigations both at the biophysical and biochemical levels.

Experimental

Melting points were determined using a Reicher Thermovar apparatus and are uncorrected. Except when otherwise mentioned NMR spectra were recorded at 200 MHz on Bruker AC 200 spectrometer using solvent as the internal reference (dimethylsulphoxide-d_6 at 2.49 ppm); the chemical shifts are reported in ppm, in δ units. The mass spectra were recorded on Varian Mat 311 and AET MS 30 instruments. High resolution mass spectra were obtained from Centre Regional de Mesures Physiques de l'Ouest, Université de Rennes. Absorption spectra were obtained on a Perkin-Elmer Lambda UV/Vis spectrometer. Microanalyses were performed by the 'Service Central de Microanalyses du CNRS', Lyon. Purifications were generally performed by chromatography on silica gel or alumina. Purifications on Sephadex LH-20 and ion exchange resins were also tested for the most polar compounds. The purity of the compounds was assessed by reversed-phase HPLC, performed on a μ-bondapak C18 analytical column (Waters Associates). The chromatographic system is equipped with two M-510 pumps and a photodiode array detector Waters 996 using Millenium 32 software. A linear gradient from 0 to 100% methanol in H_2O pH 2.5 (phosphoric acid), 2 mL/min flow rate, was used. Products were characterized by their retention time and UV/Visible absorption.

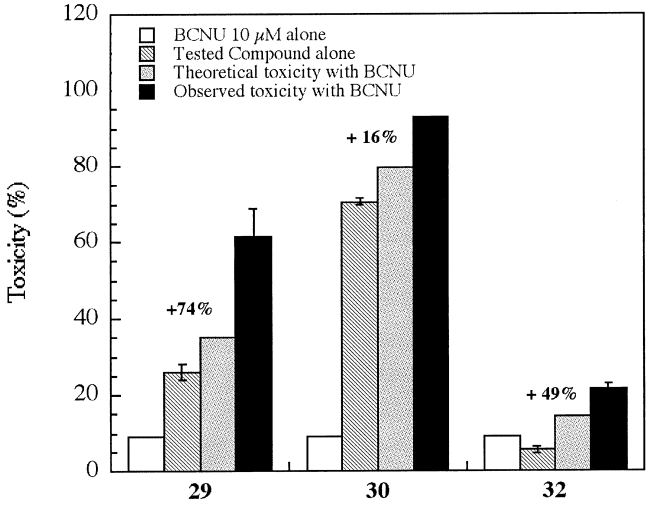


Figure 3. Apparent synergy of compounds **29**, **30** and **32** with BCNU. Apparent synergy was measured by simultaneous exposition of A549 cells to 10 μM of the tested compound and 10 μM BCNU and expressed as the % of toxicity increase with regard to the addition of the toxic effects of both drugs alone taken as 100% (theoretical toxicity).

3-(*tert*-Butoxycarbonylamino)propylamine 4 has been already described[29] **4-(*tert*-Butoxycarbonylamino)butylamine 5.** A solution of di-*tert*-butyldicarbonate (10 mL, 44 mmol) in chloroform (250 mL) was added dropwise to a solution of 1,3-diaminobutane (22 mL, 220 mmol) in chloroform (500 mL) cooled at 0 °C. The solution was stirred overnight. The unsoluble was filtered off and the solvent was removed under reduced pressure. The oily residue thus formed was diluted with cold brine (80 mL) and the white precipitate of *N*,*N*′-di(*tert*-butoxycarbonyl)diaminobutane was filtered off. Extraction of the aqueous phase with diethylether afforded after evaporation of the organic solvent the desired mono protected compound **5** as an oil in 95% yield (7.88 g, 41.8 mmol).

^{1}H NMR (200 MHz, $CDCl_3$): δ ppm 4.74 (1H, m, N*H*); 3.04 (2H, q, CH_2–NH); 2.63 (2H, t, CH_2–NH_2); 1.36 (15H, m, CH_2–CH_2–CH_2–CH_2, NH_2 and C(CH_3)$_3$)[30]

***N*-(3-(*tert*-Butoxycarbonylamino)propyl)-*N*′-trichloroethoxycarbonyl thiourea 6.** Trichloroethyl chloroformate (1.25 mL, 9.26 mmol) was added dropwise to a solution of potassium thiocyanate (1 g, 10.3 mmol) in acetone (20 mL) cooled at 0 °C. After 2 h of stirring, compound **4** was added (1.79 g, 10.3 mmol) to the solution and the resulting mixture was stirred overnight at room temperature. After evaporation of the solvent, the residue was diluted with water, the pH was adjusted to

pH 4 and compound **6** was extracted with dichloromethane. The organic layer was washed with water, dried over sodium sulfate and evaporated. Compound **6** was obtained as a yellow solid in 83% yield (3.13 g, 7.66 mmol). Mp 160 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ ppm = 9.61 (1H, m, N*H*); 8.24 (1H, m, N*H*); 4.76 (2H, s, CH_2–CCl_3); 3.73 (2H, q, CH_2–NH); 3.18 (2H, q, CH_2–NH); 1.82 (2H, quint, CH_2–CH_2–CH_2); 1.61 (1H, m, NH); 1.43 (9H, s, C(CH_3)$_3$). ^{13}C NMR (50 MHz, $CDCl_3$): δ 178.6 (C=S); 156.2 (C=O); 150.6 (C=O); 93.9 [CH_2-*C*Cl_3); 79.4 (*C*$(CH_3)_3$]; 74.7 (*C*H_2–CCl_3); 42.8 (N–*C*H_2); 37.3 (N–*C*H_2); 29.0 (CH_2-*C*H_2-CH_2); 28.3 (C(*C*H_3)$_3$). ms (CI, ammoniac + isobutane): M = 408, *m*/*z*: 408 (27, M^+); 352 (100, $M^+ - C(CH_3)_3$); 293 (72, $M^+ - CCl_3$); 234 [24, $M^+ - C(CH_3)_3$–CCl_3]. Anal. found: C, 35.44; H, 4.81; N, 10.26%; calcd for $C_{12}Cl_3H_{20}N_3O_4S$: C, 35.26; H, 4.93; N, 10.28%.

***N*-(4-(*tert*-Butoxycarbonylamino)butyl)-N′-trichloroethoxycarbonyl thiourea 7.** The procedure described above was used starting from potassium thiocyanate (0.5 g, 5.14 mmol), trichloroethyl chloroformate (0.625 mL, 4.62 mmol) and 4-(*tert*-butoxycarbonylamino) butylamine **5** (0.968 g, 5.14 mmol). Compound **7** was isolated in 86% yield (1.69 g, 3.99 mmol). Mp 140 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.44 (1H, m, N*H*); 8.28 (1H, m, N*H*); 4.75 (2H, s, CH_2–CCl_3); 4.56 (1H, s, N*H*); 3.67 (2H, q, CH_2–NH); 3.14 (2H, q, CH_2–NH); 1.69 (2H, m, CH_2–CH_2–CH_2–CH_2); 1.53 (2H, m, CH_2–CH_2–CH_2–CH_2); 1.42 (9H, s, C(CH_3)$_3$). ^{13}C NMR (50 MHz, $CDCl_3$): δ 179.0 (C=S); 156.4 (C=O); 151.4 (C=O); 94.4 (CH_2–*C*Cl_3); 79.6 [*C*$(CH_3)_3$]; 75.2 (*C*H_2–CCl_3); 45.7 (N–*C*H_2); 40.4 (N–*C*H_2); 28.7 [C(*C*H_3)$_3$]; 27.8 (*C*H_2); 25.9 (CH_2). ms (CI, ammoniac + isobutane): M = 423, *m*/*z* 424 [49, $(M+H)^+$]; 366 [100, $M^+ - C(CH_3)_3$]; 248 [47, $M^+ - C(CH_3)_3$–CCl_3]. Anal. found: C, 35.35; H, 5.02; N, 9.76%; calcd for $C_{13}H_{22}N_3O_4Cl_3S$, 0.7 H_2O, 0.05 CH_2Cl_2 C, 35.65; H, 5.39; N, 9.56%.

2,6-Diamino-9-(2-phthalimidoethyl)-9*H*-purine 9: DAP-C$_2$-Phth. *N*-(*tert*-Butyl-dimethylsilyl)phthalimide[27] (0.2 g, 0.78 mmol) was added to a suspension of 9-(2-bromoethyl)-2,6-diaminopurine[11] (0.2 g, 0.78 mmol) in THF (20 mL). Tetrabutylammonium fluoride (0.4 g, 1.1 mmol) was added in portions at room temperature and the mixture was heated at 50 °C under nitrogen for 24 h. After cooling to room temperature, the solution was filtered and the solid residue was washed several times with THF. Purification was achieved by column chromatography (silica gel, from 0 to 2% methanol in ethylacetate), **9** was then obtained in 40% yield (0.1 g, 0.3 mmol). Mp 299–300 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 7.79 (4H, m, Phth-*H*), 7.61 (1H, s, H-8); 6.55 (2H, m, NH_2); 5.37 (2H, m, NH_2); 4.21 (2H, t, DAP-CH_2); 3.91 (2H, t, CH_2-NPhth). ms [FAB (+), glycerol]: M = 323, *m*/*z* 324 $(M+H)^+$.

2,6-Diamino-9-(3-phthalimidopropyl)-9*H*-purine 10: DAP-C$_3$-Phth. Sodium hydride (600 mg, 15 mmol) was washed with pentane and then added to a suspension of 2,6-diaminopurine (2 g, 13.3 mmol) in DMF (60 mL) kept under nitrogen. After 2 h of stirring, 3-bromopropylphthalimide (4 g, 15 mmol) was added and the resulting mixture was stirred 5 days at room temperature. The solvent was evaporated to dryness and the residue was triturated in methanol–water mixture (1/1). The solid thus formed, was filtered and washed with methanol. Compound **10** was obtained in 53% yield (2.39 g, 7.1 mmol). Mp 194 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 7.83 (4H, m, Phth-*H*), 7.72 (1H, s, H-8); 6.60 (2H, m, NH_2); 5.72 (2H, m, NH_2); 3.99 (2H, t, DAP-CH_2); 3.57 (2H, t, CH_2-NPht); 2.06 (2H, quint, $CH_2CH_2CH_2$). ^{13}C NMR (75 MHz, DMSO-d_6): δ 167.8; 160.1; 156.0; 151.6; 137.4; 134.2 (2 *C*H); 131.6; 122.9 (2 *C*H); 113.2; 40.2 (N–*C*H_2); 35.0 (N–*C*H_2); 28.3 (*C*H_2). ms (CI, ammoniac + isobutane): M = 337, *m*/*z* 338 (97, $(M+H)^+$); 206 (100). Anal. found: C, 44.87; H, 4.64; N, 22.49%; calcd for $C_{16}H_{15}N_7O_2$, 1 HBr, 0.5 H_2O, 0.5 MeOH: C, 44.71; H, 4.32; N, 22.12%.

2,6-Diamino-9-(4-phthalimido-butyl)-9*H*-purine 11: DAP-c$_4$-phth. The procedure described above was used to prepare compound **11**, starting from 2,6-diaminopurine (2 g, 13.3 mmol), sodium hydride (60%, 0.6 g, 15 mmol) and 4-bromobutylphthalimide (4.14 g, 15 mmol). Compound **11** was thus obtained in 52% yield (2.42 g, 6,9 mmol). Mp 269 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 7.83 (4H, s, Pht-*H*); 7.67 (1H, s, H-8); 6.59 (2H, s, NH_2); 5.72 (2H, s, NH_2); 3.95 (2H, t, DAP–CH_2); 3.58 (2H, t, CH_2–NPht); 1.73 (2H, m, DAP-CH_2–CH_2); 1.56 (2H, m, CH_2–CH_2-NPht). ^{13}C (50 MHz, DMSO d_6): δ 167.7; 159.9; 155.8; 137.3; 134.2 (*C*H); 131.3; 122.8 (*C*H); 112.9; 104.5 (*C*H); 41.6 (N–*C*H_2); 36.7 (N-*C*H_2); 26.6 (*C*H_2); 25.0 (*C*H_2). ms [FAB(+), glycerol]: M = 351, *m*/*z* 352 [100, $(M+H)^+$]. Anal. found: C, 57.49; H, 4.82; N, 27.29%; calcd for $C_{17}H_{17}N_7O_2$, 0.2 H_2O: C, 57.52; H, 4.94; N, 27.62%.

2,6-Diamino-9-(ω-aminoalkyl)-9*H*-purine: general procedure. A solution of DAP-C$_n$-Phth (1.8 mmol) in 12N hydrochloric acid/water/acetic acid mixture (1/1/1) (6 mL) was stirred at 100 °C for 24 h. After cooling to room temperature, the solution was concentrated under reduced pressure. The phthalic acid that precipitated was filtered off. The solvent was evaporated to dryness and the residue triturated in diethylether. The solid thus formed was filtered, and (DAP-C$_n$–NH_2) were obtained as the trihydrochlorides in 70–75% yield. Products were purified by crystallization from ethanol.

12. (DAP-C$_2$–NH_2). Mp 202–205 °C. ^{1}H NMR (200 MHz, D_2O): δ 8.01 (1H, s, H-8); 4.49 (2H, t, DAP-CH_2); 3.53 (2H, m, CH_2–NH_2). ms [FAB (+), glycerol]: M = 193, *m*/*z* 194 (100, $M+H)^+$.

13. (DAP-C$_3$-NH_2). Mp 290 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.81 (2H, m, NH_2); 8.20 (3H, m, NH_2 and H-8); 7.59 (2H, m, NH_2); 4.15 (2H, t, DAP-CH_2); 2.75 (2H, m, CH_2–NH_2); 2.08 (2H, quint, $CH_2CH_2CH_2$); ms (CI, ammoniac + isobutane): M = 208, *m*/*z* 208 (100, M^+).

14. (DAP-C$_4$–NH_2): mp 277 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.11 (1H, s, H-8); 7.91 (2H, m, NH_2); 7.52 (2H, m, NH_2); 4.04 (2H, m, DAP-CH_2); 2.80 (2H, m,

CH_2–NH_2); 1.80 (2H, m, DAP-CH_2–CH_2); 1.49 (2H, m, CH_2–CH_2–NH_2). ^{13}C NMR (75 MHz, DMSO-d_6): δ 152.5; 150.5; 150.2; 140.9; 110.4; 42.5 (N–CH_2); 37.9 (N–CH_2); 26.1 (CH_2–CH_2–CH_2–CH_2); 23.7 (CH_2–CH_2–CH_2–CH_2). ms [FAB(+), glycerol]: M = 221, *m/z* 222 (100, $(M+H)^+$).

Preparation of the protected guanidines. Typical procedure

***N*-[3-(*tert*-Butoxycarbonylamino)-propyl]-*N′*-(trichloroethoxycarbonyl)-*N″*-[2-(2,6-diamino-purin-9-yl)ethyl]guanidine 15.** A mixture of thiourea **6** (0.5 g, 1.22 mmol), DAP-C_2–NH_2 **12** (0.48 g, 1.58 mmol) and EDC [N′-(3-dimethylaminopropyl)-*N*-ethyl-carbodiimide hydrochloride] (0.352 g, 1.83 mmol) was solubilized in DMF (14 mL). Triethylamine (0.663 mL, 4.76 mmol) was added to the solution and the mixture was stirred at room temperature for 4 h. The solvent was evaporated and the oily residue was diluted with water and compound **15** was extracted with dichloromethane. After evaporation of the solvent, **15** was obtained as an oil in 71% yield (0.494 g, 0.87 mmol). ^1H NMR (200 MHz, $CDCl_3$): δ 7.50 (1H, s, H-8); 5.43 (1H, m, N*H*); 4.94 (2H, s, NH_2); 4.74 (2H, s, CH_2-CCl_3); 4.25 (2H, t, DAP-CH_2); 3.74 (2H, m, CH_2); 3.17 (4H, m, 2 CH_2); 1.97–1.69 (4H, m, CH_2–CH_2–CH_2 and NH_2); 1.41 (9H, s, $C(CH_3)_3$). ms [FAB(+), glycerol]: M = 568, *m/z* 569 ($(M+H)^+$); 419 (M^+−DAP); 276 (100).

N-[4-(*tert*-Butoxycarbonylamino)-butyl]-*N′*-(trichloroethoxycarbonyl)-*N″*-[2-(2,6-diamino-purin-9-yl)ethyl]guanidine **16**, was prepared from thiourea **7** (0.250 g, 0.59 mmol) and DAP-C_2–NH_2 **12** (0.232 g, 0,77 mmol). It was obtained in 60% yield (0.207 g, 0.35 mmol). ^1H NMR (200 MHz, $CDCl_3$): δ 7.48 (1H, s, H-8); 5.50 (1H, m, N*H*); 4.82 (2H, m, NH_2); 4.74 (2H, s, CH_2–CCl_3); 4.25 (2H, t, DAP-CH_2); 3.78 (2H, m, CH_2); 3.11 (4H, m, 2 CH_2); 1.51 (4H, m, CH_2–CH_2–CH_2–CH_2); 1.40 [9H, s, $C(CH_3)_3$]. ms [FAB(+), glycerol]: M = 582, *m/z* 583 [100, $(M+H)^+$].

N-[3-(*tert*-Butoxycarbonylamino)-propyl]-*N′*-(trichloroethoxycarbonyl)-*N″*-[3-(2,6-diamino-purin-9-yl)propyl]guanidine **17**, was prepared from thiourea **6** (0.8 g, 1.96 mmol) and DAP-C_3–NH_2 **13** (0.805 g, 2.55 mmol). After the treatment described above, the oily residue was purified by column chromatography on silica gel (elution dichloromethane/methanol, 9/1, v/v mixture containing 1% ammonium hydroxide). Compound **17** was obtained as an oil in 12% yield (0.14 g, 0.24 mmol). ^1H NMR (200 MHz, $CDCl_3$): δ 9.51 (1H, m, N*H*); 7.48 (1H, s, H-8); 6.43 (1H, m, N*H*); 5.95 (2H, m, NH_2); 5.37–5.07 (3H, m, NH_2 and N*H*); 4.74 (2H, s, CH_2–CCl_3); 4.09 (2H, t, DAP-CH_2); 3.39 (2H, m, CH_2); 3.19 (4H, m, 2 CH_2); 1.99 (2H, m, CH_2–CH_2–CH_2); 1.64 (2H, m, CH_2–CH_2–CH_2); 1.38 (9H, s, $C(CH_3)_3$). ^{13}C NMR (50 MHz, CD_3OD): δ ppm 162.9; 161.9; 161.5; 159.9; 158.7; 157.5; 153.2; 139.8 (*C*H-8); 114.1 (C-5); 97.6 (CH_2–CCl_3); 80.1 ($C(CH_3)_3$); 76.4 (CH_2–CCl_3); 41.5 (N–CH_2); 39.4 (N–CH_2); 38.9 (N–CH_2); 38.5 (N–CH_2); 30.8 (CH_2); 28.8 [$C(CH_3)_3$]. ms [FAB(+), glycerol]: M = 582, *m/z* 583 [$(M+H)^+$].

N-[4-(*tert*-Butoxycarbonylamino)-butyl]-*N′*-(trichloroethoxycarbonyl)-*N″*-[3-(2,6-diamino-purin-9-yl)propyl]guanidine **18**, was prepared from thiourea **7** (0.72 g, 1.70 mmol) and DAP-C_3–NH_2 **13** (0.70 g, 2.22 mmol). Compound **18** was obtained as an oil in 79% yield (0.804 g, 1.35 mmol). ^1H NMR (200 MHz, $CDCl_3$): δ 7.53 (1H, s, H-8); 5.61 (1H, m, N*H*); 4.92 (1H, m, N*H*); 4.75 (2H, s, CH_2–CCl_3); 4.13 (2H, t, DAP-CH_2); 3.26 (4H, m, 2 CH_2); 3.11 (2H, m, CH_2); 2.33 (4H, m, 2 NH_2); 2.00 (2H, m, CH_2–CH_2–CH_2); 1.57 (4H, m, CH_2–CH_2–CH_2–CH_2); 1.39 (9H, s, $C(CH_3)_3$). ^1H NMR (200 MHz, DMSO-d_6): δ 7.72 (1H, s, H-8); 6.80 (1H, m, N*H*); 6.64 (1H, m, N*H*); 6.39 (1H, m, N*H*); 5.76 (1H, m, N*H*); 4.74 (2H, s, CH_2–CCl_3); 3.96 (2H, t, DAP-CH_2); 3.15 (4H, m, 2 CH_2); 2.91 (2H, m, CH_2); 1.93 (2H, m, CH_2–CH_2–CH_2); 1.35 [13H, m, CH_2–CH_2–CH_2–CH_2 and $C(CH_3)_3$]. ^{13}C NMR (75 MHz, DMSO-d_6): δ 160.2; 159.6; 156.8; 156.1; 155.6; 153.5; 137.4; 96.7 (CH_2–CCl_3); 77.4; 75.3; 37.7; 28.2; 26.9; 22.9. ms (FAB(+), NBA): M = 595, *m/z* 596 [$(M+H)^+$]; 447 (M^+−DAP).

N-[3-(*tert*-Butoxycarbonylamino)-propyl]-*N′*-(trichloroethoxycarbonyl)-*N″*-[4-(2,6-diamino-purin-9-yl)butyl]guanidine **19**, was prepared from thiourea **6** (0.76 g, 1.86 mmol) and DAP-C_4-NH_2 **14** (0.80 g, 2.41 mmol). After the treatment described above, the oily residue was purified by column chromatography on silica gel (elution dichloromethane/methanol, 9/1 mixture containing 1% ammonium hydroxide). Compound **19** was obtained as an oil in 12% yield (0.132 g, 0.22 mmol). Mp 79–80 °C. ^1H NMR (200 MHz, $CDCl_3$): δ 8.71 (1H, m, N*H*); 7.44 (1H, s, H-8); 6.40 (2H, broad m, NH_2); 5.27 (3H, m, NH_2 and N*H*); 4.98 (1H, m, N*H*); 4.67 (2H, s, CH_2–CCl_3); 3.93 (2H, t, DAP-CH_2); 3.28 (2H, m, CH_2); 3.19 (2H, m, CH_2); 3.07 (2H, m, CH_2); 1.80 (2H, m, CH_2–CH_2–CH_2); 1.54 (4H, m, CH_2–CH_2–CH_2–CH_2); 1.32 [9H, s, $C(CH_3)_3$].^{13}C NMR (75 MHz, CD_3OD): δ 163.1; 161.7; 161.6; 157.5; 152.7; 139.8 (*C*H-8); 114.2 (C-5); 97.6 (CH_2–CCl_3); 80.1 [$C(CH_3)_3$]; 76.4 (CH_2–CCl_3); 44.0 (N–CH_2); 41.4 (N–CH_2); 39.4 (N–CH_2); 38.5 (N–CH_2); 30.8 (CH_2); 28.8 ($C(CH_3)_3$); 28.2 (CH_2). ms [FAB(+), glycerol]: M = 596, *m/z* 597 ($(M+H)^+$); 447 (M^+−DAP). UV (ethanol 95%): λ_{max} (ε): 280 (13,000), 257 (11,000), 218 (43,900) nm.

N-[4-(*tert*-Butoxycarbonylamino)-butyl]-*N′*-(trichloroethoxycarbonyl)-*N″*-[4-(2,6-diamino-purin-9-yl)butyl]guanidine **20**, was prepared from thiourea **7** (0.7 g, 1.65 mmol) and DAP-C_4-NH_2 **14** (0.71 g, 2.14 mmol). After the treatment described above, the oily residue was purified by column chromatography on silica gel (elution dichloromethane/methanol, 9/1 mixture containing 1% ammonium hydroxide). Compound **20** was obtained as an oil in 22% yield (0.225 g, 0.36 mmol). Mp 92–95 °C. ^1H NMR (200 MHz, $CDCl_3$): δ 7.47 (1H, s, H-8); 6.30 (2H, broad m, NH_2); 5.24–5.00 (3H, m, NH_2 and N*H*); 4.69 (2H, s, CH_2–CCl_3); 3.96 (2H, t, DAP-CH_2); 3.19 (4H, m, 2 CH_2); 3.05 (2H, m, CH_2); 1.80 (2H, m, CH_2–CH_2–CH_2–CH_2); 1.46 (6H, m, CH_2–CH_2–CH_2–CH_2 and CH_2–CH_2–CH_2–CH_2); 1.34 (9H, s, $C(CH_3)_3$). ^{13}C NMR (50 MHz, CD_3OD): δ 163.1; 161.7; 161.5; 158.6; 152.7; 139.9 (*C*H-8); 114.2; 100.0; 97.6 (CH_2–CCl_3); 79.9 [$C(CH_3)_3$]; 76.5 (CH_2–

CCl_3); 44.0 (N–CH_2); 41.8 (N–CH_2); 41.4 (N–CH_2); 40.8 (N–CH_2); 28.8 (C(CH_3)$_3$); 28.3 (CH_2); 28.1 (CH_2); 27.5 (CH_2). ms [FAB(+), NBA]: M = 609, *m/z* 609 (M^+); 461 (M^+ + 1-DAP).

One-pot deprotection of the guanidine and the primary amine. General procedure

A suspension of zinc powder (1.4 g) in acetic acid/water (5/1, v/v) mixture (3.5 mL) was cooled at 0 °C. Boc-, Troc-protected compound (**15**–**20**) was added and the resulting mixture was stirred for 4 h. The suspension was then filtered onto Celite to remove zinc and salts. The solvent was evaporated and the oily residue was diluted in hydrochloric acid 1 N in acetic acid. After 1 h of stirring at room temperature, the solvent was evaporated under reduced pressure. The fully deprotected compounds (**21**–**26**) were obtained as tetrahydrochlorides. They appeared as very hygroscopic gums and they were used without further purification. The purity was checked by hplc (purity ⩾95%), and the total disappearance of Boc and Troc protecting groups was assessed by ^{1}H NMR.

***N*-(3-Aminopropyl)-*N″*-[2-(2,6-diamino-purin-9-yl)ethyl]-guanidine 21. 31% yield.** ^{1}H NMR (200 MHz, DMSO-d_6): δ 7.98 (1H, d, H-8); 7.68 (7H, m, 2 NH_2 and N*H*); 4.15 (2H, m, DAP-CH_2); 3.55 (2H, m, CH_2); 3.15 (2H, m, CH_2); 2.79 (2H, m, CH_2); 1.71 (2H, m, CH_2–CH_2–CH_2).

***N*-(4-Aminobutyl)-*N″*-[2-(2,6-diamino-purin-9-yl)ethyl]-guanidine 22. 30% yield.** ^{1}H NMR (200 MHz, DMSO-d_6): δ 7.81 (1H, s, H-8); 7.66 (7H, m, NH_2 and N*H*); 7.53 (1H, m, N*H*); 4.12 (2H, t, DAP-CH_2); 3.55 (2H, m, CH_2); 3.10 (2H, m, CH_2); 2.77 (2H, m, CH_2); 1.49 (4H, m, CH_2–CH_2–CH_2–CH_2). ^{1}H NMR (200 MHz, D_2O): δ 7.71 (1H, s, H-8); 4.11 (2H, t, DAP-CH_2); 3.49 (2H, t, CH_2); 2.84 (4H, m, 2 CH_2); 1.41 (2H, m, CH_2–CH_2–CH_2–CH_2); 1.26 (2H, m, CH_2–CH_2–CH_2–CH_2).

***N*-(3-Aminopropyl)-*N″*-[3-(2,6-diamino-purin-9-yl)propyl]guanidine 23. 54% yield.** ^{1}H NMR (300 MHz, D_2O): δ 7.96 (1H, s, H-8); 3.99 (2H, t, DAP-CH_2); 3.20 (4H, t, 2 CH_2); 2.84 (2H, m, CH_2); 1.97 (2H, m, CH_2–CH_2–CH_2); 1.77 (2H, m, CH_2–CH_2–CH_2).

***N*-(4-Aminobutyl)-*N″*-[3-(2,6-diamino-purin-9-yl)propyl]-guanidine 24.** ^{1}H NMR (200 MHz, D_2O): δ 7.67 (1H, d, H-8); 4.03 (2H, m, DAP-CH_2); 3.07 (2H, m, CH_2); 2.84 (4H, m, 2 CH_2); 2.00 (2H, m, CH_2–CH_2–CH_2); 1.41 (4H, m, CH_2–CH_2–CH_2–CH_2).

***N*-(3-Aminopropyl)-*N″*-[4-(2,6-diamino-purin-9-yl)butyl]-guanidine 25. 41% yield.** ^{1}H NMR (200 MHz, D_2O): δ 7.79 (1H, s, H-8); 3.90 (2H, t, DAP-CH_2); 3.06 (2H, t, CH_2); 2.98 (2H, t, CH_2); 2.84 (2H, t, CH_2); 1.71 (4H, m, CH_2–CH_2–CH_2–CH_2); 1.38 (2H, m, CH_2–CH_2–CH_2).

***N*-(4-Aminobutyl)-*N″*-[4-(2,6-diamino-purin-9-yl)butyl]-guanidine 26.** The presence of large amount of salts in the final product and its high hygroscopic character precluded accurate NMR analysis.

Final coupling with the acridine nucleus. Typical procedure

***N*-[3-(6-Chloro-2-methoxy-acridin-9-yl-amino)propyl]-*N″*-[2-(2,6-diamino-purin-9-yl)ethyl]-guanidine DAP-C2-gua-C3-NHAcr 27.** A mixture of **21** (0.095 g, 0.22 mmol), triethylamine (0.12 mL, 0.88 mmol) and 6-chloro-2-methoxy-9-phenoxyacridine (0.145 g, 0.44 mmol) was stirred in phenol (2 mL) at 80 °C under nitrogen for 5 h. The solution was slowly poured into acetone (25 mL) under vigorous stirring. The yellow solid was filtered and washed carefully with acetone. The solid was solubilized in water and addition of 1N sodium hydroxide allowed compound **27** to precipitate as a free base. After filtration, the solid was washed several times with water. Crystallization from methanol containing 12 N hydrochloric acid and acetone afforded **27** as the tetrahydrochloride in 9% yield (0.013 g, 0.02 mmol). Mp 220–230 °C. ^{1}H NMR (200 MHz, CD3OD) (free base): δ 8.16 (1H, d, *J* = 9.6 Hz, Acr-H); 7.76 (1H, s, H-8); 7.71 (2H, m, Acr-H); 7.44 (1H, d, *J* = 2.4 Hz, Acr-H); 7.33 (1H, dd, *J* = 9.2 and 2.4 Hz, Acr-*H*); 7.20 (1H, dd, *J* = 9.2 and 2.1 Hz, Acr-*H*); 4.07 (2H, t, DAP-CH_2); 3.89 (3H, s, O–CH_3); 3.80 (2H, t, CH_2); 3.42 (2H, t, CH_2); 3.12 (2H, t, CH_2); 1.91 (2H, m, CH_2–CH_2–CH_2). ms (CI, ammoniac + isobutane): M = 533, *m/z* 534 (2, $(M+H)^+$); 244 (76, $(Acr+1)^+$). UV (H_2O): λ_{max} (ε): 444 (7100), 423 (7400), 343 (3800), 279 (44,500), 217 (36,500) nm. hrms [FAB(+), NBA]: found: 534.2248; calcd for $C_{25}ClH_{28}N_{11}O$ + H: 534.2245.

***N*-[4-(6-Chloro-2-methoxy-acridin-9-yl-amino)butyl]-*N″*-[2-(2,6-diamino-purin-9-yl)ethyl]-guanidine DAP-C_2-gua-C_4–NHAcr 28.** Compound **22** (0.113 g, 0.25 mmol), triethylamine (0.14 mL, 1.0 mmol) and 6-chloro-2-methoxy-9-phenoxyacridine (0.167 g, 0.50 mmol) were mixed in phenol (3 mL). The procedure was the same as described above. 51% yield. mp: 210–215 °C. ^{1}H NMR (200 MHz, D_2O): δ 8.03 (1H, d, *J* = 9.6 Hz, Acr-*H*); 7.60 (1H, s, H-8); 7.30 (5H, m, Acr-*H*); 3.94 (2H, t, DAP-CH_2); 3.77 (3H, s, O–CH_3); 3.55 (2H, m, CH_2); 3.21 (2H, m, CH_2); 3.12 (2H, t, CH_2); 1.84 (2H, m, CH_2–CH_2–CH_2–CH_2); 1.62 (2H, m, CH_2–CH_2–CH_2–CH_2). ^{13}C NMR (50 MHz, D_2O): δ 155.8; 155.5; 154.3; 151.6; 151.3; 148.9; 142.0; 140.6; 138.4; 127.1; 124.4; 124.3; 119.8; 116.7; 112.9; 108.8; 102.3; 56.3 (O–CH_3); 47.4 (NH–CH_2); 41.7 (NH–CH_2); 40.6 (NH–CH_2); 40.5 (NH-CH_2); 26.5 (CH_2–CH_2–CH_2–CH_2); 25.6 (CH_2–CH_2–CH_2–CH_2). ms (FAB(+), NBA): M = 547, *m/z*: 548 ($(M+H)^+$). UV (H_2O): λ_{max} (ε): 423 (6000), 279 (38500), 217 (31300) nm. hrms [FAB(+), NBA]: found: 548.2420; calcd for $C_{26}ClH_{30}N_{11}O$ + H: 548.2402.

***N*-[3-(6-Chloro-2-methoxy-acridin-9-yl-amino)propyl]-*N″*-[3-(2,6-diamino-purin-9-yl)propyl]guanidine DAP-C_3-gua-C_3NHAcr 29.** The procedure was the same as the typical procedure described above, except the purification step. After filtration, the solid obtained from the precipitation in acetone was dissolved in water. The pH was adjusted to pH 12 sodium hydroxide. As **29** did not precipitate from the alkaline solution, the aqueous phase was washed several times with ethyl acetate, and extracted with butanol. The organic layer was washed with water, dried on sodium sulfate and evaporated

under reduced pressure. The oily residue was dissolved in methanol, the solution was filtered, and 12 N hydrochloric acid was added to it. Compound **29** precipitated by adding acetone/diethylether (1/1 mixture) to the solution and was isolated as tetrahydrochloride in 42% yield (0.111 g, 0.16 mmol). mp: 210–220 °C. ^{1}H NMR (300 MHz, D_2O): δ 7.97 (1H, d, J = 9.3 Hz, Acr-*H*); 7.64 (1H, s, Acr-*H*); 7.34–7.24 (4H, m, Acr-*H* and H-8); 7.17 (1H, d, J = 9.3 Hz, Acr-*H*); 4.01 (2H, m, DAP-CH_2); 3.77 (3H, s, O–CH_3); 3.57 (2H, m, CH_2); 3.34 (2H, m, CH_2); 2.95 (2H, m, CH_2); 2.14 (2H, m, CH_2–CH_2–CH_2); 1.67 (2H, m, CH_2–CH_2–CH_2). ^{13}C NMR (75 MHz, D_2O): δ 156.5; 155.9; 154.4; 152.3; 151.6; 149.6; 142.3 (*C*H); 141.2; 139.4; 134.0; 127.6 (*C*H); 124.8 (*C*H); 120.5 (*C*H); 117.4 (*C*H); 113.7; 110.9; 109.6; 102.9; 102.8 (*C*H); 56.6 (O–*C*H_3); 46.5 (N–*C*H_2); 40.7 (N–*C*H_2); 39.7 (N–*C*H_2); 39.1 (N–*C*H_2); 28.2 (*C*H_2); 28.0 (*C*H_2). ms (CI, ammoniac + isobutane): M = 547, m/z 548 [$(M+H)^+$]. UV (H_2O): λ_{max} (ε): 424 (7100), 343 (3800), 281 (46,800), 217 (53,200) nm.

***N*-[4-(6-Chloro-2-methoxy-acridin-9-yl-amino)butyl]-*N″*-[3-(2,6-diamino-purin-9-yl)propyl]guanidine DAP-C_3-gua-C_4-NHAcr 30.** Compound **30** was prepared as described above for **27**. The free base was purified by chromatography on acidic alumina (elution dichloromethane/methanol, 8/2 followed by 6/4 mixtures). The fractions containing **30** were collected and evaporated to dryness. The residue was contaminated with alumina. It was dissolved in water and **30** was extracted with butanol. The solvent was evaporated under reduced pressure. **30** was crystallized from methanol containing 12 N hydrochloric acid by adding diethylether. It was thus isolated as the tetrahydrochloride in 24% yield (0.215 g, 0.32 mmol). Mp: 210–220 °C. ^{1}H NMR (200 MHz, CD_3OD) (Free base): δ 8.20 (1H, d, J = 9.2 Hz, Acr-*H*); 7.77 (1H, s, H-8); 7.72 (2H, m, Acr-*H*); 7.46 (1H, d, J = 1,7 Hz, Acr-*H*); 7.32 (1H, dd, J = 9.2 Hz and 2.4 Hz, Acr-*H*); 7.20 (1H, dd, J = 9.2 Hz and 2.1 Hz, Acr-*H*); 3.99 (2H, t, DAP-CH_2); 3.89 (3H, s, O–CH_3); 3.79 (2H, t, CH_2); 3.01 (4H, m, 2 CH_2); 1.90 (4H, m, 2 CH_2); 1.45 (2H, m, CH_2). ^{13}C NMR (50 MHz, D_2O): δ 155.8; 155.6; 154.0; 151.4; 150.8; 148.7; 141.8; 140.6; 132.9; 127.5; 127.0; 124.5; 119.8; 116.8; 112.6; 110.0; 56.2 (O–*C*H_3); 47.8 (NH–*C*H_2); 40.5 (NH–*C*H_2); 40.4 (NH–*C*H_2); 38.0; 26.5; 25.6. ms [FAB(+), glycerol]: M = 561, m/z 562 [$(M+H)^+$]. UV (H_2O): λ_{max} (ε): 423 (6000), 278 (36900), 217 (32900) nm. hrms [FAB(+), NBA]: found: 562.2552; calcd for $C_{27}ClH_{32}N_{11}O$ + H: 562.2558.

***N*-[3-(6-Chloro-2-methoxy-acridin-9-yl-amino)propyl]-*N″*-[4-(2,6-diamino-purin-9-yl)butyl]guanidine DAP-C_4-gua-C_3-NHAcr 31.** Compound **31** was prepared and purified with the methodology described for **29**. It was isolated as the tetrahydrochloride in 25% yield (0.013 g, 0.018 mmol). Mp 185–190 °C. ^{1}H NMR (300 MHz, D_2O): δ 7.80 (1H, d, J = 9.3 Hz, Acr-*H*); 7.59 (1H, s, Acr-*H*); 7.28–7.25 (3H, m, Acr-*H* and H-8); 7.13–7.09 (2H, m, Acr-*H*); 3.87 (2H, t, DAP-CH_2); 3.74 (3H, s, O–CH_3); 3.66 (2H, t, CH_2); 3.20 (2H, t, CH_2); 2.92 (2H, t, CH_2); 2.01 (2H, m, CH_2–CH_2–CH_2); 1.49 (2H, m, CH_2–CH_2–CH_2–CH_2); 1.34 (2H, m, CH_2–CH_2–CH_2–CH_2). ^{13}C NMR (75 MHz, D_2O): δ 156.4; 155.9; 155.7; 153.3; 150.9; 150.5; 141.8; 141.0; 139.3; 133.9; 127.4; 127.3; 124.5; 120.4; 117.4; 113.6; 111.0; 109.3; 102.8; 56.5 (O–*C*H_3); 46.5 (NH–*C*H_2); 43.5 (NH–*C*H_2); 40.7 (NH–*C*H_2); 38.6 (NH–*C*H_2); 28.6 (CH_2–*C*H_2–CH_2); 26.6 (CH_2–*C*H_2–CH_2); 25.1 (CH_2–*C*H_2–CH_2). ms (FAB(+), glycerol): M = 561, m/z 562 [$(M+H)^+$]. UV (H_2O): λ_{max} (ε): 423 (6200), 279 (41,800), 217 (36,000) nm. hrms [FAB(+), NBA]: found: 562.2556; calcd for $C_{27}ClH_{32}N_{11}O$ + H: 562.2558.

***N*-[4-(6-Chloro-2-methoxy-acridin-9-yl-amino)butyl]-*N″*-[4-(2,6-diamino-purin-9-yl)butyl]guanidine DAP-C_4-gua-C_4-NHAcr 32.** Compound **32** was prepared and purified with the methodology described for **29**. It was isolated as the tetrahydrochloride in 37% (0.011 g, 0.015 mmol). Mp 210–215 °C. ^{1}H NMR (300 MHz, D_2O): δ 7.81 (1H, d, J = 9.3 Hz, Acr-*H*); 7.50 (1H, s, Acr-*H*); 7.30–7.25 (3H, m, Acr-*H* and H-8); 7.14 (1H, dd, J = 9.3 and 2.0 Hz, Acr-*H*); 7.09 (1H, d, J = 2.0 Hz, Acr-*H*); 3.78 (2H, m, DAP-CH_2); 3.74 (3H, s, O–CH_3); 3.56 (2H, t, CH_2); 3.17 (2H, t, CH_2); 3.03 (2H, t, CH_2); 1.79 (2H, m, CH_2–CH_2–CH_2–CH_2); 1.60 (2H, m, CH_2–CH_2–CH_2–CH_2); 1.49 (2H, m, CH_2–CH_2–CH_2–CH_2); 1.37 (2H, m, CH_2–CH_2–CH_2–CH_2). ^{13}C NMR (75 MHz, D_2O): δ ppm 156.2; 156.1; 155.3; 153.1; 150.9; 150.3; 141.6; 140.9; 139.2; 134.0; 128.9; 127.3; 124.7; 120.4; 117.4; 113.3; 110.9; 109.4; 56.5 (O–*C*H_3); 48.3 (NH–*C*H_2); 43.3 (NH–*C*H_2); 40.8 (NH–*C*H_2); 40.5 (NH–*C*H_2); 26.9 (CH_2–CH_2–*C*H_2–CH_2); 26.3 (CH_2–*C*H_2–CH_2–CH_2); 25.7 (CH_2–CH_2–*C*H_2–CH_2); 25.0 (CH_2–*C*H_2–CH_2–CH_2). ms [FAB(+), glycerol]: M = 575, m/z 576 [$(M+H)^+$]. UV (H_2O): λ_{max} (ε): 423 (5000), 278 (32,100), 217 (29,900) nm.

DNA binding studies

DNA binding experiments were performed with Perkin Elmer MPF-44A and LS50 spectrofluorimeters in a thermostated quartz cell (25 °C). Excitation and emission were monitored at the ethidium bromide bands (520 and 600 nm). All solutions were made in 25 mM Tris–HCl, pH 7.0, 0.1 M NaCl and 0.2 mM EDTA buffer. Calf-thymus DNA was used. DNA concentration was 1.6×10^{-5} M (in base pairs) and that of tested drug 2×10^{-5} M.

In vitro assays. L1210 cells (Mouse leukemia, ATCC CCL 219) were cultivated in Dulbecco's MEM supplemented with 10% fetal calf serum. L1210 cells were seeded at 10^5 cells/mL in 1 mL microwell plates. After 24 h (usually 3 to 4×10^5 cells/mL) tested compounds were added in duplicate at various concentrations and incubated for 24 h. Cells were counted with a Coulter-Counter ZM (Coultronics Inc.). The dose inhibiting the growth by 50% (IC_{50}) was interpolated from regression curves obtained with experimental points without significant toxicity.

A549 cells (human pulmonary adenocarcinoma, ATCC CCL 185) were grown in Kaighn modified Ham F12 medium (F12-K) supplemented with 10% fetal calf serum. Cells were plated in 3 cm diameter multiwell

plates (200 cells/well). After 24 h, tested compounds, alone or with 10 μM BCNU, were added in duplicate at various concentrations and cells were incubated for another 24 h. Cells were washed with phosphate buffered saline (PBS) and then reincubated for 14 days with fresh medium free of drug. Colonies were numbered after washing with PBS and staining with Giemsa. Survival was expressed as% of untreated controls and IC_{50} was interpolated from regression curves obtained with experimental points.

All incubations were carried out at 37 °C in water-jacketed CO_2 incubator (5% CO_2, 100% relative humidity).

The purity of tested compounds was assessed by HPLC and was $\geq$98%.

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