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A Charge-Deficient Analogue of Spermine with Chelating Properties

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Abstract—1,12-Diamino-3,6,9-triazadodecane, a new isosteric and charge-deficient analogue of spermine, is synthesized. Unlike spermine, the new analogue is an excellent chelator of Cu^{2+} ions. Possible applications of this compound for studying enzymes of polyamine metabolism and cellular functions of spermine are discussed.

Key words: DNA, polyamines, spermine

INTRODUCTION

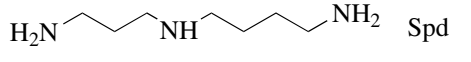
The biogenic polyamines spermine, spermidine, and putrescine are present in significant amounts in almost all cell types and may be considered as universal low-molecular regulators of cellular metabolism [1].[2] Biological effects of polyamines and their analogues are determined by the geometry of molecule that provides for the proper spatial arrangement of amino groups, the degree of their protonation, and their ability to form a network of hydrogen bonds while interacting with specific cellular binding sites. The majority of efforts to correlate the biological activity of polyamine analogues with their structures were devoted to the study of structural analogues of Spm and Spd, including the derivatives with various substituents at the terminal nitrogen atoms and analogues with conformationally restricted fragments in the polyamine backbone (see reviews [2, 3]).

Investigations of the contribution of protonation of the Spm/Spd amino groups to the biological effects of polyamines are more limited. The analogues with pyridinium cycle incorporated into its backbone [4] and 2,2-, 6,6-, and 7,7-difluorospermidines were synthesized [5] in order to decreased the basicity of their amino groups. The isosteric charge-deficient analogues of Spd [6] and Spm [7] were obtained by substitution of aminooxy (H_2NO) groups with pK_a ~5 for the terminal aminomethylene groups of Spd and Spm. These compounds turned out to be effectors of the enzymes of polyamine metabolism. They are capable of penetration into various cell types and possess a comparatively low cytotoxicity and a controlled catabolic stability [8–10]. An oxa analogue of Spd and a dioxa analogues of Spm containing HNO group(s) instead of the central $HNCH_2$ group(s) were also synthesized [11]; however, their biochemistry has insufficiently been investigated up to now.

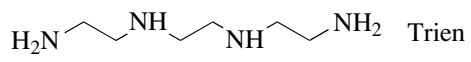
The present paper is devoted to the principles of design and synthesis of a new isosteric Spm analogue with a charge-deficient central fragment, which, unlike Spm, is an excellent chelator for Cu^{2+} ions.

RESULTS AND DISCUSSION

Trien, whose backbone includes three $–CH_2CH_2–$ groups, may be considered as an isosteric analogue of Spd. A reduced to two methylene units distance between secondary amino groups results in a strong decrease in the basicity of secondary amino groups, whereas the pK_a values of terminal amino groups remain almost the same as in parent Spd [12].



pK_a	10.9	8.4	9.9	



pK_a	9.9	6.7	3.3	9.2

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[2] Abbreviations: Ms, methanesulfonyl; Spd, spermidine (1,8-diamino-4-azaoctane; Spm, spermine (1,12-diamino-4,9-diazadodecane); SpmTrien, 1,12-diamino-3,6,9-triazadodecane; and Trien, triethylenetetramine.

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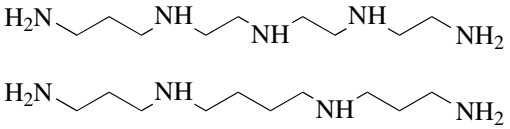
Trien can effectively penetrate into cells, and Spm inhibits its uptake [13]. Unlike Spd, Trien is an effective chelator of bivalent cations of transition metals and, due to an excellent stability of its complex with Cu^{2+} (pK_{dis} 20.4 at pH 14 and 14.0 at pH 7 [14]), Trien is used for the treatment of Wilson's disease at daily doses up to 1.2–1.6 g [15], which indicates its low toxicity.

The replacement of the Spd fragment in the Spm molecule by a triethylenetetramine moiety gives rise to earlier unknown SpmTrien.



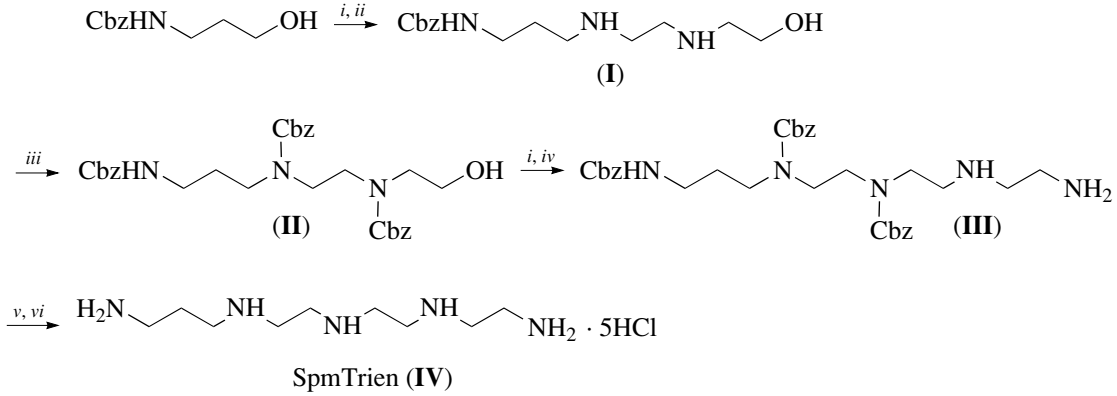
SpmTrien can be considered as an isoster of Spm; however, unlike Spm, its molecule is asymmetric. SpmTrien is charge-deficient in respect of its central fragment, since its pK_a (N^3, N^6) < 7 and only three of its amino groups are protonated at physiological pH value. The presence of triethylenetetramine fragment provides SpmTrien with good chelating properties toward the cations of transition metals. Note that neither Spm nor its analogues possess such a combination of properties.

Thus, SpmTrien could be used in biochemical studies of polyamines: in the investigation of the peculiarities of Spm active transport into cells; as a new chemical regulator of the enzymes of polyamine catabolism; and for studying the polyamine interaction with nucleic acids. The combination of properties of SpmTrien imply that its Spm-like effects may be at least pH-dependent at the interaction with the specific sites of Spm binding. The ability or inability of SpmTrien to support the cells growth with the depleted polyamine pool may be considered as a criterion of its integral biochemical similarity or dissimilarity to Spm.

A rational strategy of the SpmTrien synthesis consists in a successive elongation of the polyamine analogue backbone by alkylation of the corresponding free amines with appropriate active esters of alcohols. Such an approach was used to prepare several polyamine analogues (see [16,17] and references therein), and we successfully used it previously to synthesize the aminooxy analogue of Spm [7] and also α-methyl-Spd [18].

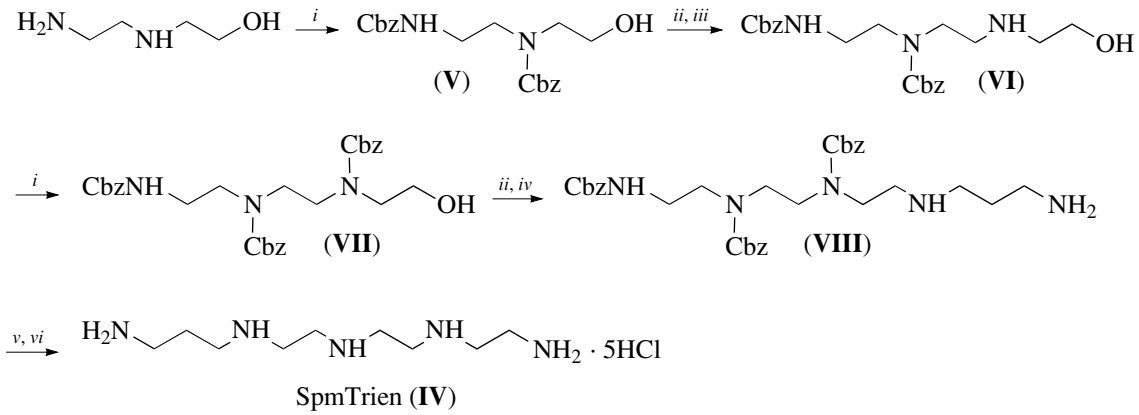
The first approach to the preparation of SpmTrien (Scheme 1) envisages the build up of its polyamine backbone starting from a three carbon fragment, *N*-benzyloxycarbonyl-3-aminopropanol.

It was converted into the corresponding mesylate, which was treated without purification with an excess *N*-(2-aminoethyl)-aminoethanol in THF. The unreacted *N*-(2-aminoethyl)-aminoethanol was rapidly removed in a vacuum of oil pump, the residue was dissolved in 1 M NaOH, extracted with dichloromethane, and, after a flash chromatography on silica gel, crude (**I**) was obtained. This appeared to contain the alkylation product of the secondary amino group of *N*-(2-aminoethyl)-aminoethanol as a minor hardly removable impurity. The crude mono benzyloxycarbonylamino (**I**) were acylated with benzyl chloroformate, which led, after flash chromatography on silica gel, to pure trisbenzyloxycarbonyl derivative (**II**). (**II**) was converted into mesylate, which was treated without purification with excess ethylenediamine in THF. The ethylenediamine excess was distilled off in a vacuum, and the subsequent flash chromatography of the residue on silica gel gave trisbenzyloxycarbonyl-SpmTrien (**III**). The protective groups were removed from (**III**) by catalytic hydrogenation over Pd-black in a methanol–acetic acid



Reagents: *i*, $MsCl/Et_3N/CH_2Cl_2$; *ii*, $H_2NCH_2CH_2NHCH_2CH_2OH/THF$;
iii, $CbzCl/NaHCO_3/THF/H_2O$; *iv*, $H_2NCH_2CH_2NH_2/THF$;
v, $H_2/Pd/AcOH/MeOH$; *vi*, HCl/MeOH.

Scheme 1.



Reagents: *i*, $CbzCl/NaHCO_3/THF/H_2O$; *ii*, $MsCl/Et_3N/CH_2Cl_2$; *iii*, $H_2NCH_2CH_2OH/THF$; *iv*, $H_2NCH_2CH_2CH_2NH_2/THF$; *v*, $H_2/Pd/AcOH/MeOH$; *vi*, HCl/MeOH.

Scheme 2.

mixture, and the resulting SpmTrien pentaacetate was converted into pentahydrocloride.

The second approach to the synthesis of SpmTrien envisages the build up of the analogue backbone starting from a two-carbon fragment (Scheme 2). This approach allowed the avoidance of the ambiguously proceeding stage of the primary amino group alkylation in the presence of unprotected secondary amino group.

Methodologically, this scheme is similar to the first approach and also is a five-stage process. However, the isolation and purification procedures for the intermediates are less laborious. The overall yields of SpmTrien (**IV**) were ~20% in both cases.

It is known that Spm and terminally asymmetric bisalkylated polyamine analogues can protect DNA from the free-radical damage under the conditions of Fenton reaction [19]. This property of Spm was proved to be due to its direct reaction with free radicals [20]. SpmTrien is an isosteric analogue of Spm and, in the case of free radicals generated in the system H_2O_2/Cu^{2+}, SpmTrien protects DNA far better than Spm (Fig.1).

One can see from Fig. 1 that, at 25 μM concentration and the molar ratio SpmTrien–Cu^{2+} of 1.25 : 1, SpmTrien effectively prevents the degradation of pUC19 DNA (lane *5*) and a twofold increase in the SpmTrien concentration hardly improves the protection of DNA (lane *6*). The content of DNA circular form at 25 μM concentration of SpmTrien is much lower then that in the presence of 1 mM Spm (cf. lanes *5* and *4* in Fig. 1). The observed differences in the protective properties of Spm and SpmTrien seem not to be due to the differences in their DNA-binding properties or to the reactivity as free radical scavengers. A more likely explanation of the inhibition of DNA damage is the formation of stable complex between SpmTrien and Cu^{2+}, which affects the production of free radicals in the Cu^{2+}/H_2O_2 system.

A greater complexation capacity of SpmTrien for Cu^{2+} ions than that of Spm was confirmed in another model. SpmTrien demonstrated the ability to destroy complex three-dimensional molecular constructs formed on the basis of linear double-stranded DNA molecules fixed within the spatial structure of cholesteric liquid-crystalline dispersion and crosslinked by planar polymer bridges. These consisted of alternating daunomycin molecules and Cu^{2+} ions [21], which is schematically presented in Fig. 2.

According to the results of work [21], the formation of such molecular constructs is accompanied by the appearance of an extra intense band in CD spectrum in the region of daunomycin absorption (Fig. 3a, curve *2*). Obviously, the elimination of Cu^{2+} ions from the polymeric chelate bridge as a result of introduction into the system of such an effective chelator of Cu^{2+} ions as SpmTrien (Fig. 4) would lead to the destruction of the polymer bridge and, consequently, to the disappearance of the anomal band in the CD spectrum of the DNA

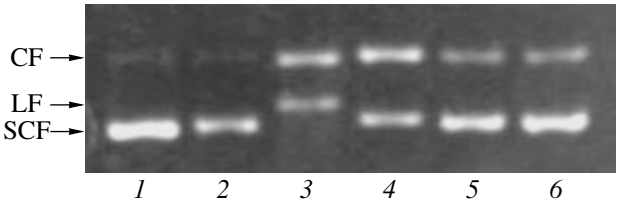


Fig. 1. Electrophoresis in 0.7% agarose gel of the degradation products of supercoiled DNA pUC19 effected by the H_2O_2/Cu^{2+} treatment. Lanes: *1*, pUC19; *2*, pUC19 + PBS, 60 min, 37°C; *3*, pUC19 + PBS + 20 μM $CuCl_2$ + 30 μM H_2O_2, 60 min, 37°C; *4*, the same as *3* + 1 mM Spm; *5*, the same as *3* + 25 μM SpmTrien; *6*, the same as *3* + 50 μM SpmTrien. CF, circular; LF, linear; and SCF, supercoiled form of DNA, respectively.

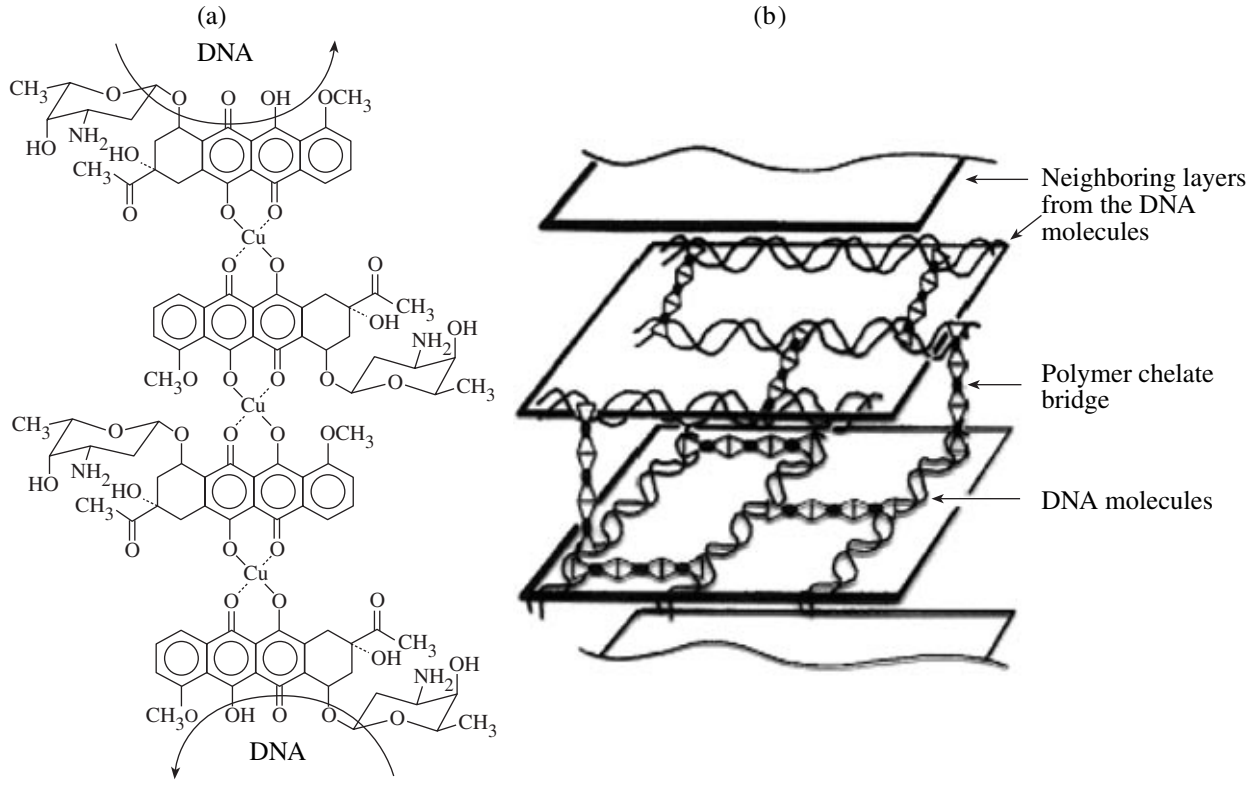


Fig. 2. (a) Schematic representation of polymeric crosslink between two neighboring DNA molecules (the view along the long axis of DNA molecule) and (b) a hypothetical structure of molecular construct (in accordance with [21]) prepared from DNA cholesteric liquid-crystalline dispersion.

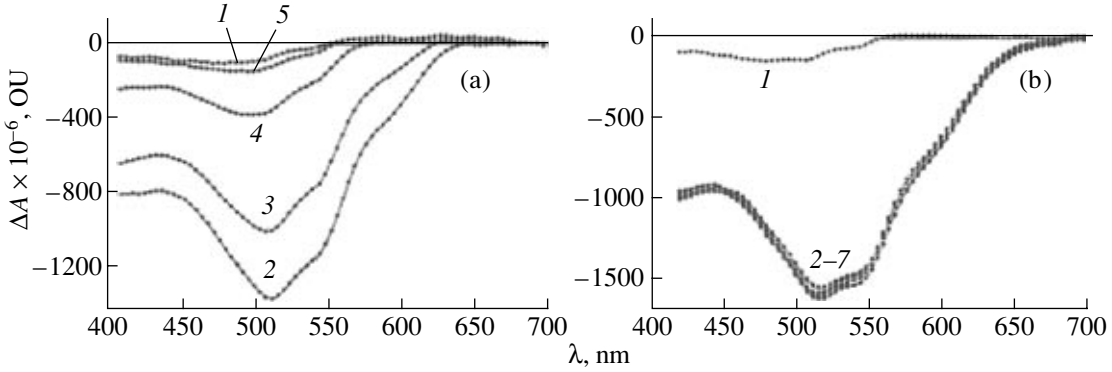


Fig. 3. CD spectra of DNA molecular construct (curve *2*) before and (a, curves *3–5*) after treatment with SpmTrien and Spm (b, curves *3–7*). 1, DNA (5.6 μg/ml, daunomicyn (2.7×10^{-5} M), PEG_{4000} (170 mg/ml) in 0.01 M sodium phosphate buffer (pH 7) containing 0.3 M NaCl (the CD spectrum of cholesteric liquid-crystalline dispersion of DNA–daunomycin complex); *2*, the same as *1* but in the presence of 1×10^{-5} M $CuCl_2$; (a) the same as *2*, but in the presence of *3*, 2.5×10^{-6} M; *4*, 4.9×10^{-6} M; and *5*, 7.3×10^{-6} M SpmTrien; (b) the same as *2*, but in the presence of *3*, 2.5×10^{-6} M; *4*, 4.9×10^{-6} M; *5*, 1.5×10^{-5} M, *6*, 2.4×10^{-5} M; and *7*, 4.9×10^{-5} M Spm.

molecular construct. Therefore, the amplitude of this band can be used as an analytical criterion that allows a comparison of Cu^{2+}-chelating properties of SpmTrien and Spm.

Treatment of the DNA molecular construct with SpmTrien at concentration of 7.3×10^{-6} M at a SpmTrien–Cu^{2+} molar ratio of 0.7 : 1 resulted in disappearance of the anomal band in the CD spectrum

(Fig. 3a, curve *5*). The interaction of SpmTrien with DNA could not be the reason of the CD spectral changes, since the Spm 2.5×10^{-6}–4.9×10^{-5} M concentrations exert no effect (Fig. 3b, curves *3*–*7*). Therefore, the observed changes in CD spectrum were due to a strong complexation of SpmTrien with Cu^{2+}.

Thus, the presence of triethylenetetramine fragment in the SpmTrien molecule results in appearance in this isosteric analogue of an ability to effectively chelate Cu^{2+} ions. Such a property is new for Spm analogues. A unique combination of central fragment charge deficiency of central fragment with significant chelating properties makes SpmTrien and its derivatives a useful tool for the investigation of polyamine cellular functions and enzymes of their metabolism.

EXPERIMENTAL

3-Aminopropanol, ethanolamine, ethylenediamine, 1,3-diaminopropane, *N*-(2-aminoethyl)-aminoethanol, benzyloxycarbonyl chloride, and mesyl chloride were purchased from Fluka (Switzerland); polyethyleneglycol 4000 Da from Ferak (Germany); and Spm, daunomycin, Tris-base, ethidium bromide, Na_2-EDTA, and agarose from Sigma (United States). Other reagents were manufactured in Russia. *N*-Benzyloxycarbonyl-3-aminopropanol was prepared as described in [22].

Supercoiled DNA pUC19 was isolated from *Escherichia coli* strain DH5α, which was transformed with pUC19 DNA, and purified by a CsCl-gradient centrifugation as described in [23]. Chicken erythrocyte DNA with molecular mass of ~$(0.3–0.7) \times 10^6$ Da was from Reanal (Hungary); it was additionally purified from trace proteins and low-molecular polysaccharides by the procedure [24]. Concentrations of DNA and daunomycin were spectrophotometrically determined in aqueous salt solutions, using the molar absorption coefficients ε_{260} 6600 M^{-1} cm^{-1} [23] and ε_{475} 12000 M^{-1} cm^{-1} [25], respectively. The DNA-containing molecular construct was prepared as previously described in [26, 27].

TLC was carried out on precoated Kieselgel 60 F_{254} plates (Merck, Germany) using the following elution systems: (A) 9 : 1 chloroform–methanol, (B) 9 : 1 dioxane–25% ammonia, (C) 95 : 5 chloroform –methanol, (D) 95 : 5 dioxane–25% ammonia, (E) 97 : 3 dioxane–25% ammonia, and (F) 4 : 2 : 1 : 2 *n*-butanol–acetic acid–pyridine–water. Kieselgel (40–63 μm, Merck, Germany) was used for column chromatography; elution systems are indicated in the text. TLC spots of compounds were visualized in UV-light and also using color reaction with ninhydrin.

Absorption spectra were registered using Specord M40 (Germany), and CD-spectra using a portable dichrometer SKD-2 (Institute of Spectroscopy, Russian Academy of Sciences, Troitzk, Moscow oblast). ^1H and ^{13}C NMR spectra were measured on a Bruker Avance 500 DRX (Germany) at the working frequency of 500.1 MHz for protons and 125.8 MHz for ^{13}C nuclei in $CDCl_3$ (if other is not indicated) using TMS ($CDCl_3$) and sodium 3-trimethylsilylpropanesulfonate (D_2O) as internal standards. Chemical shifts are given in ppm, and constants of spin–spin interaction in Hz.

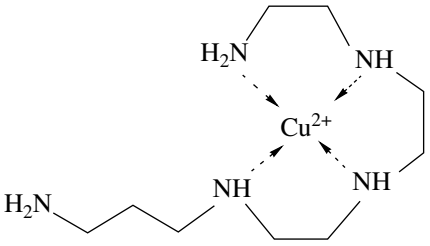


Fig. 4. The structure of Cu^{2+}–SpmTrien complex.

9-(Benzyloxycarbonylamino)-3,6-diazanonan-1-ol (I). A solution of MsCl (0.87 ml, 0.011 mol) in dry dichloromethane (10 ml) was added for 15 min to a stirred and cooled (0°C) solution of *N*-Cbz-3-aminopropanol (2.09 g, 0.01 mol) and Et_3N (2.08 ml, 0.015 mol) in dry dichloromethane (50 ml). Stirring was continued for 1 h at 0°C and for 1 h at 20°C. The reaction mixture was poured into 1 M $NaHCO_3$ (30 ml); the organic layer was separated, successively washed with water (10 ml), 0.5 M H_2SO_4 (3 × 30 ml), water (20 ml), 1 M $NaHCO_3$ (30 ml), and water (2 × 10 ml); dried with $MgSO_4$; and evaporated in a vacuum to dryness. The residue was dissolved in THF (25 ml), cooled to 0°C, and freshly distilled *N*-(2-aminoethyl)-aminoethanol (20.0 ml, 0.2 mol) was added in one portion. The reaction mixture was kept for 24 h at 20°C, THF and unreacted amine were then distilled off in a vacuum. The residue was poured into 2 M NaOH (20 ml); extracted with dichloromethane (2 × 20 ml); organic layer was separated, washed with water (2 × 3 ml); dried with K_2CO_3 and evaporated in a vacuum. The residue was dissolved in MeOH–Et_3N (8 : 2, 10 ml), applied onto a silica gel column (125 g) and eluted with 8 : 2 MeOH–Et_3N. The fractions containing (**I**) were evaporated in a vacuum and dried in a vacuum over P_2O_5. This resulted in (**I**); a viscous oil; yield 1.8 g (60%); R_f 0.18 (B); ^1H NMR: 7.35–7.24 (5 H, m, C_6H_5), 5.70 (1 I, bs, NHCbz), 5.07 (2 H, c, CH_2Ph), 3.62–3.54 (2 H, m, C$\underline{H}_2$OH), 3.30–3.20 (2 H, m, C$\underline{H}_2$NHCbz), 2.75–2.62 (6 H, m, C$\underline{H}_2$NH), 2.57–2.47 (2 H, m, C$\underline{H}_2$NH), 2.20 (3 H, bs, NH + OH), 1.68–1.58 (2 H, m, $CH_2C\underline{H}_2CH_2$).

***N*^{3},*N*^{6},*N*^{9}-Tris(benzyloxycarbonyl)-9-amino-3,6-diazanonan-1-ol (II).** Benzyl chloroformate (1.5 ml, 12 mmol) was added in three portions at 15-min intervals to a cooled (0°C) and vigorously stirred mixture of (**I**) (1.5 g, 5 mmol), THF (10 ml), water (3 ml), $NaHCO_3$ (0.84 g, 10 mmol), and 10 M NaOH (0.3 ml). Stirring was continued for 1 h at 0°C and then for 3 h at

20°C, organic layer was separated, the aqueous phase was extracted with dichloromethane (2 × 5 ml), and the combined organic layers were evaporated to dryness in a vacuum. The residue was dissolved in dichloromethane (20 ml); the solution was successively washed with 0.5 M HCl (3 × 10 ml), water (2 × 10 ml), and 0.5 M $NaHCO_3$ (2 × 10 ml); dried with $MgSO_4$; and evaporated in a vacuum. The residue was dissolved in system (C) (7 ml) and applied onto a silica gel column (125 g), which was eluted with system (C) to give 2.4 g of crude (**II**). This was again purified on the same column eluted with 96 : 4 chloroform–methanol mixture to give (**II**); a viscous oil; yield 1.9 g (68%); R_f 0.45 (C); ^{1}H NMR: 7.40–7.20 (15 H, m, C_6H_5), 5.48 (1 H, bs, NHCbz), 5.17–4.98 (6 H, m, CH_2Ph), 3.75–3.53 (2 H, m, C$\underline{H}_2$OH), 3.52–2.95 (10 H, m, CH_2NCbz), 2.36 (1 H, bs, OH), 1.61–1.45 (2 H, m, $CH_2C\underline{H}_2CH_2$).

***N*^{6},*N*^{9},*N*^{12}-Tris(benzyloxycarbonyl)-1,12-diamino-3,6,9-triazadodecane (III).** A solution of MsCl (0.25 ml, 3.2 mmol) in dry dichloromethane (5 ml) was added for 20 min to a stirred and cooled (0°C) solution of (**II**) (1.7 g, 3 mmol) and Et_3N (0.7 ml, 5 mmol) in dry dichloromethane (20 ml). Stirring was continued for 1 h at 0°C and for 1 h at 20°C. The reaction mixture was poured into 1 M $NaHCO_3$ (10 ml); the organic layer was separated, washed with water (8 ml), 0.5 M H_2SO_4 (3 × 7 ml), water (8 ml), 1 M $NaHCO_3$ (5 ml), and water (2 × 10 ml); dried with $MgSO_4$; and evaporated in a vacuum. The residue was dissolved in dry THF (10 ml), cooled to 0°C, and ethylenediamine (4 ml, 60 mmol) was added in one portion. The reaction mixture was kept for 14 h at 20°C, and THF and unreacted diamine were then distilled off in a vacuum. The residue was dissolved in 8 : 2 methanol–Et_3N (10 ml) and the resulting solution was separated into two parts, each part was purified on a silica gel column (125 g) eluted with the same solvent mixture to get (**III**); a viscous oil after drying in a vacuum over P_2O_5; yield 1.07 g (59%); R_f 0.20 (D); ^{1}H NMR: 7.42–7.22 (15 H, m, C_6H_5), 5.53 (1 H, bs, NHCbz), 5.17–4.97 (6 H, m, CH_2Ph), 3.47–2.93 (10 H, m, CH_2NCbz), 2.85–2.43 (6 H, m, C$\underline{H}_2$NH), 1.60–1.19 (5 H, m, $CH_2C\underline{H}_2CH_2$ + N$\underline{H}$ + N$\underline{H}_2$).

***N*^{3},*N*^{5}-Bis(benzyloxycarbonyl)-5-amino-3-azapentan-1-ol (V).** Benzyl chloroformate (15 ml, 0.11 mol) was added in five portions with 20-min intervals to a cooled (0°C) and vigorously stirred mixture of *N*-(2-aminoethyl)-aminoethanol (5.2 g, 0.05 mol), THF (100 ml), water (17 ml), $NaHCO_3$ (8.4 g, 0.1 mol), and 10 M NaOH (2.5 ml). Stirring was continued for 1 h at 0°C and for 3 h at 20°C. Organic layer was separated and evaporated to dryness in a vacuum. The residue was dissolved in EtOAc (100 ml); the solution was successively washed with 1.0 M HCl (3 × 40 ml), water (2 × 40 ml); dried with $MgSO_4$; and evaporated in a vacuum. The residue was dissolved in chloroform (25 ml) and applied onto a silica gel column (300 g) eluted first with chloroform and then with a system (A). The fraction of (**V**) was evaporated and dried over P_2O_5 in a vacuum; yield 13 g (70%); a viscous oil, which solidified on storage; R_f 0.30 (A); ^{1}H NMR: 7.40–7.20 (10 H, m, C_6H_5), 5.53 (0.5 H, bs, NHCbz), 5.31 (0.5 H, bs, NHCbz), 5.06 (2 H, c, CH_2Ph), 5.04 (2 H, c, CH_2Ph), 3.71 (2 H, m, C$\underline{H}_2$OH), 3.50–3.25 (6 H, m, CH_2N), 2.86 (1 H, bs, OH).

***N*^{6},*N*^{8}-Bis(benzyloxycarbonyl)-8-amino-3,6-diazaoctan-1-ol (VI).** A solution of MsCl (0.61 ml, 7.8 mmol) in dry dichloromethane (5 ml) was added for 10 min to a stirred and cooled (0°C) solution of (**V**) (2.65 g, 7.1 mmol) and Et_3N (1.15 ml, 8.3 mmol) in dry dichloromethane (20 ml). Stirring was continued for 1 h at 0°C and for 1 h at 20°C. The reaction mixture was treated with 1 M $NaHCO_3$ (20 ml); the organic layer was separated, washed with 0.5 M H_2SO_4 (3 × 15 ml), water (10 ml), 1 M $NaHCO_3$ (20 ml), and water (10 ml); dried with $MgSO_4$; and evaporated to dryness in a vacuum. The residue was dissolved in dry THF (7 ml), cooled to 0°C, and ethanolamine (4.2 ml, 68 mmol) was added in one portion. The reaction mixture was kept for 6 h at 20°C and 48 h at 20°C, and THF and unreacted amine were distilled off in a vacuum. The residue was dissolved in 2 M NaOH (10 ml) and extracted with chloroform (2 × 7 ml); organic layer was separated, washed with water (2 × 2 ml); dried with K_2CO_3 and evaporated in a vacuum. The residue was dissolved in 100 : 0.8 dioxane–25% ammonia (10 ml); applied onto a silica gel column (130 g) and eluted with the same solvent mixture. The resulting (**VI**) was dried in a vacuum over P_2O_5; yield 2.05 g (70%); R_f 0.39 (E); ^{1}H NMR: 7.34–7.26 (10 H, m, C_6H_5), 5.70 (1 I, bs, NHCbz), 5.10 (2 H, s, CH_2Ph), 5.05 (2 H, s, CH_2Ph), 3.58–3.52 (2 H, m, C$\underline{H}_2$OH), 3.43–3.34 (6 H, m, CH_2NCbz), 2.83–2.64 (4 H, m, C$\underline{H}_2$NH), 2.25 (2 H, bs, NH + OH).

***N*^{3},*N*^{6},*N*^{8}-Tris(benzyloxycarbonyl)-8-amino-3,6-diazaoctan-1-ol (VII).** Benzyl chloroformate (0.8 ml, 5.4 mmol) was added in two portions at a 20-min interval to a cooled (0°C) and vigorously stirred mixture of (**VI**) (1.95 g, 4.7 mmol), THF (6 ml), water (2 ml), $NaHCO_3$ (0.7 g, 8.3 mmol), and 10 M NaOH (0.3 ml). Stirring was continued for 1 h at 0°C and then for 3 h at 20°C. Organic layer was separated, the aqueous phase was extracted with dichloromethane (5 ml), and the combined organic layers were evaporated to dryness in a vacuum. The residue was dissolved in chloroform, dried with $MgSO_4$, and evaporated in a vacuum. The residue was dissolved in 99 : 1 chloroform–methanol (15 ml); applied onto a silica gel column (130 g) and eluted with the same solvent mixture. This resulted in (**VII**); yield 2.19 g (85%); R_f 0.6 (C); ^{1}H NMR: 7.34–7.23 (15 H, m, C_6H_5), 5.10–4.98 (6 H, m, CH_2Ph), 3.75–3.63 (2 H, m, C$\underline{H}_2$OH), 3.54–3.05 (10 H, m, CH_2NCbz), 2.36 (1 H, bs, OH).

***N*^{1},*N*^{3},*N*^{6}-Tris(benzyloxycarbonyl)-1,12-diamino-3,6,9-triazadodecane (VIII).** A solution of MsCl (0.3 ml, 3.8 mmol) in dry dichloromethane (5 ml) was added for 10 min to a stirred and cooled (0°C) solution of (**VII**) (1.95 g, 3.5 mmol) and Et_3N (0.55 ml, 4 mmol) in dry dichloromethane (20 ml). Stirring was continued for 1 h at 0°C and for 1 h at 20°C. Reaction mixture was treated with 1 M $NaHCO_3$ (5 ml); the organic layer was separated, successively washed with 0.5 M H_2SO_4 (3 × 4 ml), water (4 ml), 1 M $NaHCO_3$ (5 ml), and water (3 ml); dried with $MgSO_4$; and evaporated in a vacuum. The residue was dissolved in dry THF (5 ml), cooled to 0°C, and 1,3-diaminopropane (2.5 ml, 30 mmol) was added in one portion. The reaction mixture was kept for 6 h at 0°C, and then 24 h at 20°C; and THF and unreacted diamine were then distilled off in a vacuum. The residue was dissolved in 1 M NaOH (10 ml) and extracted with chloroform (20 ml). The organic layer was separated, washed with water (2 × 3 ml), dried with K_2CO_3 and evaporated in a vacuum. The residue was dissolved in system (E) (10 ml); applied onto a silica gel column (130 g) and eluted with the same system. The resulting (**VIII**) was dried in a vacuum over P_2O_5; yield 1.5 g (71%); R_f 0.15 (E); ^{1}H NMR: 7.40–7.25 (15 H, m, C_6H_5), 5.10–4.98 (6 H, m, CH_2Ph), 3.49–3.05 (10 H, m, CH_2NCbz), 2.85–2.46 (6 H, m, $C\underline{H}_2NH$), 1.72 (3 H, s, NH), 1.65–1.44 (2 H, m, $CH_2C\underline{H}_2CH_2$).

1,12-Diamino-3,6,9-triazadodecane pentahydrochloride (IV). Method A. Approximately 1 ml of a suspension of Pd-black in methanol was added to a solution of (**III**) (0.97 g, 1.7 mmol) in 1 : 1 AcOH–methanol mixture (20 ml) and hydrogenation at atmospheric pressure was continued until the end of CO_2 evolution (~2.5 h). The catalyst was filtered off and washed with methanol, and the combined filtrates were evaporated in a vacuum. The residue was dissolved in hot methanol (14 ml), and after the addition of 5 M HCl (3.5 ml) and hot EtOH (15 ml) the mixture was left overnight at 0°C. The precipitate was filtered off, washed with cold 1 : 1 methanol–ethanol mixture and dried in a vacuum over P_2O_5/KOH to give (**IV**) pentahydrochloride; yield 0.52 g (79%); mp.: 265–266°C (decomp.); R_f 0.24 (F); MS, *m*/*z*: $[M + H]^+$ 204.05 ($C_9H_{25}N_5$; calculated $[M + H]^+$ 204.22); ^{1}H NMR (D_2O): 3.58–3.47 (10 H, m, $C\underline{H}_2NH$), 3.42 (2 H, t, *J* 6.9, $C\underline{H}_2NH$), 3.26 (2 H, t, *J* 8.0, $C\underline{H}_2NH$), 3.13 (2 H, t, *J* 7.5, $C\underline{H}_2NH$), 2.14 (2 H, m, $CH_2C\underline{H}_2CH_2$); ^{13}C NMR (D_2O): 48.09, 47.58, 46.85, 46.73, 46.51, 39.44, 38.50, 26.69. Found, %: C 27.87, H 7.97, N 18.10. $C_9H_{30}N_5Cl_5$. Calculated, %: C 28.03, H 7.84, N 18.16.

Method B. Approximately 0.5 ml of a suspension of Pd-black in methanol was added to a solution of (**VIII**) (0.42 g, 0.7 mmol) in 1 : 1 AcOH–methanol mixture (10 ml), and hydrogenation at atmospheric pressure was carried out until the end of CO_2 evolution (~1.5 h). The catalyst was filtered off and washed with methanol, and the combined filtrates were evaporated in a vacuum. The residue was dissolved in hot methanol (9 ml), and 5 M HCl (1.4 ml, 7 mmol) and hot EtOH (20 ml) were added. After cooling, the solution was evaporated in a vacuum to one half of initial volume, then boiling EtOH (15 ml) was added, and the resulting mixture was left at –20°C overnight. The precipitate was filtered, washed with cooled EtOH, and dried in a vacuum over P_2O_5/KOH. The yield of (**IV**) pentahydrochloride 0.19 g (70%), the preparation was identical to the sample obtained by method A.

Protection of supercoiled DNA from the free-radical H_2O_2/Cu^{2+} damage by Spm and SpmTrien (IV). A supercoiled DNA plasmid pUC19 (200 ng) was incubated for 60 min at 37°C in PBS (pH 7.4), containing 30 μM H_2O_2 and 20 μM $CuCl_2$ in total volume 30 μl (mixture A). The incubation in PBS without addition of H_2O_2 and $CuCl_2$ was used as a control. The protective effects of Spm and SpmTrien were investigated incubating mixture A containing either 1 mM Spm or SpmTrien at a concentration of 25 or 50 μM for 60 min at 37°C. Aliquots (5 μl) of the reaction mixtures were analyzed by electrophoresis in 0.7% agarose gel, containing 40 mM Tris-acetate buffer (pH 7.4) and 1 mM EDTA-Na_2. Gel was stained with ethidium bromide (2 μg/ml) for 10 min, the stain was washed out with water, and gel was photographed in UV-light. The results are presented at Fig. 1.

Interaction of SpmTrien (IV) and Spm with DNA-containing molecular constructs. DNA cholesteric liquid-crystalline dispersion was prepared in accordance with the procedure [26, 27], mixing an equal volume of DNA solution (11.2 μg/ml) in 0.01 M sodium phosphate buffer (pH 7) containing 0.3 M NaCl (buffer B) with a PEG_{4000} solution (340 mg/ml) in the same buffer B. Then, 4 mM solution of daunomycin in buffer B (13.7 μl per 2 ml of DNA cholesteric liquid-crystalline dispersion) was added at an effective stirring. A cholesteric liquid-crystalline dispersion of DNA–daunomycin complex formed. This dispersion was supplemented with 1 mM $CuCl_2$ (20 μl) in buffer B at effective stirring, which gave rise to the required three-dimensional molecular construct (detailed protocols for the preparation of DNA-containing molecular construct are described in [26, 27]). Neighboring DNA molecules in it are crosslinked with polymeric Cu^{2+}-containing polymer chelate bridges (Fig. 2b). The CD-spectra of this molecular construct were registered at the area of daunomycin absorption (Fig. 3a, curve *2*). The above solution of molecular construct (2 ml) was mixed with 1-μl portions of aqueous 4.9 mM SpmTrien solution or 4.9 mM Spm solution (1-, 2-, 5-, 10- or 20-μl portions) and, CD-spectra were measured after each addition (Fig. 3a, curves *3*–*5*). The increase in the volume of starting solution was neglected.

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