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Clustered Ergot Alkaloids Modulate Cell-mediated Cytotoxicity

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Abstract—Dimers of agroclavine (**1**) and terguride (**2**), as well as a series of terguride oligomers, for example trimers (**5**, **6**), tetramer (**7**), hexamer (**8**) and functionalized tergurides for further complex clustering were synthesized. Terguride oligomers were screened for their direct cellular toxicity on lymphoma cell lines in vitro and for their immunomodulating activities, represented by the natural killer (NK) cell-mediated cytotoxicity, as the most sensitive screening marker during immune responses. Dimers linked via aromatic spacer showed a high toxicity (1 μM) to lymphoma cells, which was not detected in other derivatives. In vitro and ex vivo experiments performed on mouse spleen lymphocytes in the presence of terguride oligomers demonstrated an immunosuppressive effect of dimers with aromatic spacer (**4c**–**d**) and NK cell stimulatory effect of terguride hexamer (**8**) and trimer with aliphatic spacer (**5c**). There is a considerable evidence that indolic part of molecule contributes to immunosuppressive action of terguride, which is potentiated in dimers carrying aromatic linker. This effect can be reversed by higher oligomerization of the respective alkaloids.

Introduction

Ergot alkaloids can serve as a physiologic agonists and/or antagonists at the α-adrenergic, dopamine, and serotonin receptors.[1] Number of compounds from this group have been tested using psychopharmacological and neurochemical assays. These compounds were tested for neuroendocrine effects, but rarely for immunological parameters. The ergoline action on immune system has been devoted predominantly to the immunosuppressive effect of D_2-dopaminergic and prolactin inhibitory ergopeptines (bromokryptine, lisuride, terguride), however some of them exhibit also strong cytostatic[2] or immunostimulating[3–5] activities. It is well known that ergot alkaloids and their derivatives display diverse pharmacological effects. Ergoline compounds act upon the neuroendocrine system by interaction with neurotransmitter membrane receptors.[1] Some ergot alkaloids exhibit also strong cytostatic[2] or immunostimulating[3–5] activities. Terguride is clinically used semisynthetic derivative of ergot alkaloid, manifesting mostly partial D_2-dopamine agonistic, but also 5-HT, and α_1-adrenergic agonistic properties. It is generally used in therapy of neuroendocrine disorders, pituitary and other prolactine producing tumors in respect to their dopamine agonistic and prolactine inhibitory properties and primarily in the treatment of Parkinson's disease.[6–8] The immunomodulatory effects, particularly the modulation of natural killer (NK) cell effector functions, can be derived from the close relation to the cells of neuroendocrine compartment, which are the primary targets of ergolines. NK cells are important part of immunosurveillance against invading pathogens, control of tumor growth.[11] They are susceptible to pituitary hormones, neurotransmitters and related compounds.[12,13] This makes them a suitable target for screening of immunomodulatory properties of any new drug.

In our previous experiments, we found also important role of terguride in the process of the recognition of tumor cells and modulation the activity of natural killer (NK), as well as specific cytotoxic T lymphocytes (CTL).[9,10] Terguride, when tested in mixed lymphocyte-tumor cultures (MLTC) in vitro showed an enhanced resistance of tumor cells to lysis and immunosuppressive effect on NK cell function.[10] From this point of view, we were interested whether the clustering of the compound could decrease the negative action of terguride. Multivalent effects are well known to improve various

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ligand binding to cell surface receptors. It is well documented that clustered haptens[14] have substantially higher affinity towards the respective receptors than their single molecules, as well as changes in pharmacodynamic (metabolism, internalization) could occur. The receptor physiology of oligomeric or dendrimeric structure combines the properties of larger molecule together with high number of haptens on its surface as well the role of spacers used for the clustering.[15] The binding properties, interaction or internalization of alkaloids (haptens) by the cells could be strongly enhanced by so called 'clustering-effect', well known, for example, in glycoconjugates.[14,15]

In this study, simple ergoline alkaloid agroclavine (**1**) was used as a model compound for the synthesis and a representative of 8,9-ergolenes. However, our attention was focused to terguride[16] (**2**) in respect to its multiple effects on neuroendocrine and immune systems. We present here synthesis of dimers and oligomers of **1** and **2** and also terguride with appropriate reactive spacers for further clustering. Terguride dimers and oligomers were tested for their direct toxic effect on lymphoid tumor cell lines and used in NK cell-mediated cytotoxicity assay in vitro. Analogous experiments were performed on mice spleen cells after in vivo application of terguride derivatives.

Results and Discussion

Large number of chemical modifications of ergot derivatives have been prepared in our and other laboratories to achieve compounds with more selective and more specific effects. However, the oligomerization of ergot alkaloids is a new approach aimed (i) at partial exposition (masking) of terguride molecule recognized by specific receptor site, or (ii) potentiation of cellular response depending on the size of molecule. Cluster effect known from, for example glycoclusters, can also be expected.

Ergot alkaloid oligomer synthesis

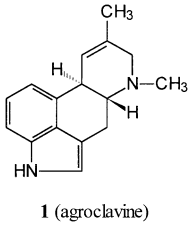
An obvious site for EA oligomerization is the indolic nitrogen. Series of new alkaloid derivatives was prepared through the alkylation in this position. Phase transfer catalysis is used mostly for industrial alkaloid *N*-methylation.[17] Some *N*-1-alkyl derivatives of agroclavine and festuclavine were prepared by alkylation by primary alkylhalogenides in a liquid NH_3 in the presence of Na.[18]

Bifunctional alkylbromides (e.g., 1,6-dibromohexane) were used for alkylation of **1** and **2** under phase transfer conditions (tetrabutylammonium hydrogen sulfate/$NaOH/CH_2Cl_2$) but no product (in the case of **2**) or insufficient amount of inseparable product mixture (in the case of **1**) was formed. No reaction was observed in DMF or DMSO when NaH was used as base, however, with powdered NaOH,[19] fast product formation was observed. The reaction was optimized (DMSO, KOH, rt) and analytical yields of respective dimers were almost quantitative. However, this method is less suitable for ergolenes ($\Delta^{8,9}$ and $\Delta^{9,10}$) where unidentified by-products are formed. In the case of **2** and other ergolines (with saturated D-ring), virtually no by-products or decomposition under given conditions was observed.

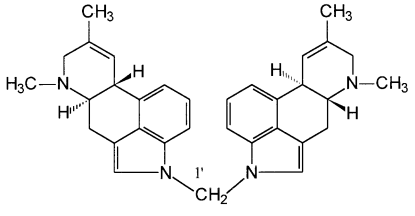
Dichloromethane was used as the simplest bifunctional spacer. Both **1** and **2** gave corresponding *N*,*N*-dimers **3a** and **4a** as determined by MS and NMR. Here, obviously carbene is formed that reacts quickly with two molecules of EA. In the case when large excess (10 equiv) of CH_2Cl_2 is used no monosubstituted product is formed. Chloroform and iodoform do not react and mono- and bifunctional arylhalogenides, for example, *p*-dibromobenzene gave no products under above conditions probably due to low reactivity.

Series of larger bifunctional bromides was used for dimerization of **2**, for example, 1,6-dibromohexane, *o*-, *p*- and *m*-bis(bromomethyl)benzenes[20]—they all gave excellent yields of respective dimers **4b–e** within short reaction time (0.5–2 h).

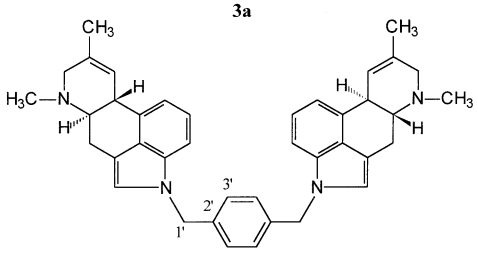
For the preparation of EA oligomer, we used as an aliphatic core pentaerythritol tetrabromide. The reaction was performed with an excess of **2** to achieve full sub-



1 (agroclavine)



3a



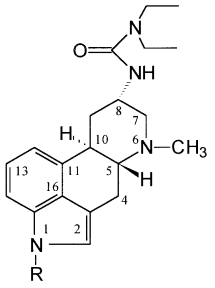
3b

stitution of the core. Despite long reaction time (20 h) no tetrasubstituted pentaerythritol—probably due to the sterical reasons—was obtained, however, mono-, di- and trisubstituted oligomers (**5a**–**c**) were isolated in quite good yields. These compounds carry one, two or three unreacted Br groups thus they could be used for further clustering.

Fully substituted EA-trimer (**6**) was obtained from symmetric core 1,3,5-trimethyl-2,4,6-tris(bromomethyl)-benzene. Due to higher reactivity of bromobenzyl groups, no partly substituted core was observed. Analogously also tetramer (**7**) was obtained using 1,2,4,5-tetrakis(bromomethyl)benzene and hexamer (**8**) using hexakis(bromomethyl)benzene. In the last case larger excess of **2** and prolonged reaction time (over 24 h) should be used to achieve full substitution. Smaller amounts of penta- and tetrasubstituted core as judged from MS (data not given) were also formed.

NMR spectra of all oligomers exhibited the expected symmetry. The substitution of indolic NH was confirmed by the absence of its proton signal (and the coupling to H-4a), and ^3J couplings of spacer CH_2 (in **3a** and **4a**) to C-2 and C-15 (and vice versa, H-2 to this methylene). Marked chemical shift changes were observed for H-2 and H-14 (in some cases accompanied by ring current effects of the aromatic ring contained in the spacer), and also for C-2, C-3, and C-14 of the ergoline moieties (Tables 1 and 2). The structures were further confirmed by ESI-MS and by elemental analysis.

For the construction of more complex alkaloid oligomers and for the dendrimer synthesis series of terguride derivatives carrying at the N-1 position, suitable functionalities were prepared under the above conditions. Allybromide afforded *N*-1-allylderivative (**9a**) for (co-)-polymerization, ethyl bromoacetate was used for preparation of *N*-1-carboxymethylderivative (**9b**) for the peptide coupling and a large excess (10 equiv) of 1,6-dibromohexane afforded ω-bromo-*N*-1-hexylterguride (**9c**). Here, only a small amount (<10%) of dimer **4b** was formed as a side product. Attempted reaction with bromoacetonitrile gave low yield of unseparable product mixture. Spacer with amino group, for example 2-amino-*N*-1-ethylterguride (**9d**) was prepared under analogous conditions using 2-bromoethylamine. This compound will mainly serve to the coupling to polymeric carriers (e.g., bromocyanosepharose) useful for affinity chromatography. N-1 Hydroxymethylterguride (**9e**) was prepared by condensation with formaldehyde.[21] This compound will be used for ester coupling and for further functionalisation (e.g., oxidation).



2 R = H (terguride)
9a R = -$CH_2CH{=}CH_2$
9b R = -CH_2COOH
9c R = -$(CH_2)_6Br$
9d R = -$CH_2CH_2NH_2$
9e R = -CH_2OH

Direct toxicity assay on tumor cell lines

From our previous experiments, it was evident that interaction of terguride with tumors and with lymphocytes could be mediated not only through specific (neurotransmitter) receptors, but mainly by direct internalization into the cytoplasmic and nuclear com-

Table 1. Direct cellular toxicity of terguride oligomers on YAC-1 and K562 tumor cell lines expessed as % of spontaneous ^{51}Cr release[a]

Compound	K562 cells	YAC1 cells
2	100	100
4a	50	105
4b	138	148
4c	750	596
4d	252	483
4e	300	624
5b	276	618
5c	70	150
6	120	89
7	77	51
8	8	27

[a]The release of ^{51}Cr by 10^4 cells/well/250 μL was measured after 18 h of incubation in the presence of tested compounds. Values are expressed in % of spontaneous release from radiolabeled cells cultivated in medium only (generally 5–7% of totally incorporated ^{51}Cr) expressed as 100%. Results represent three separate experiments in which standard deviation does not exceed 5%.

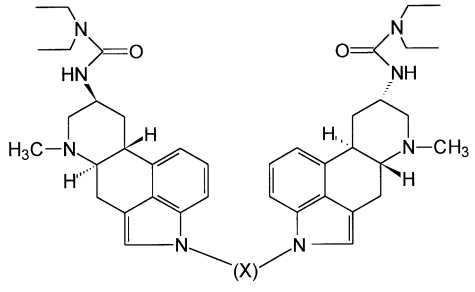
Table 2. The effect of terguride clusters on NK cell-mediated cytotoxicity in vitro

Compound	Concentration (M)		
	10^{-8}	10^{-9}	10^{-10}
a. Terguride dimers with aliphatic spacers[a]			
None (control)	100	100	100
2	113	127	143
4a	94	136	129
4b	119	120	139
5b	58	129	148
b. Terguride dimers with aromatic spacers[b]			
None (control)	100	100	100
2	113	127	143
4c	51	101	107
4d	59	124	146
5e	61	104	149
c. Terguride multivalent clusters[b]			
None (control)	100	100	100
2	113	127	143
5c	128	176	168
6	89	127	157
7	74	158	125
8	79	136	152

[a]Results are expressed as % of control values (in the absence of tested compounds), where control equals 100%, that is 6.75% of specific cytotoxicity, in E/T ratio 64:1. Experimental values represent average of six separate experiments each assayed in tetraplicates with standard deviation between 3 and 5%.
[b]See Table 2a.

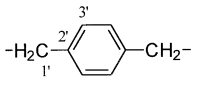
partment of cells. This was a reason for testing the direct toxicity of newly synthesized terguride clusters on established tumor cell lines in vitro. YAC-1 (mouse T lymphoma) and K562 (human erythroleukemia) cell lines were chosen for these experiments, since the same cells are generally used as tumor targets for NK cell-mediated cytotoxic activity. The level of cell damage after 18 h of incubation (37 °C, 5% CO_2) in the presence of terguride clusters and terguride itself was measured by release of ^{51}Cr from labeled cells.

The effects of terguride clusters (Table 1) were tested in the concentration range from 10^{-6} to 10^{-10} M. We have observed that terguride dimers with aromatic linker (**4c–e**) in the concentration 1 μM significantly increase the release of ^{51}Cr from the cytoplasm due to increased membrane permeability and subsequently caused cell death. Higher clustering (**6–8**) of terguride decreased the toxicity of the compounds. As shown in the Table 1, the toxicity of terguride dimers (**4c**, **4d**, **4e**) could be related to the aromatic part of the molecule, where the cell damage is plausibly dependent on the exposition of aromatic linker (**4c**). On the other hand, the terguride dimers coupled with aliphatic spacer (**4a–b**) do not exhibit any toxic effects. Similarly, in tri- and tetra-clusters of terguride (**6**, **7**), even if coupled to the aromatic nucleus, the toxicity might be lowered by a number of peripheral terguride motifs masking the aromatic ring. Moreover, the hexa-cluster of terguride (**8**) decreased the spontaneous ^{51}Cr-release to 8% (K562) or 26% (YAC-1) of controls exerting cytoprotective effects. We can assume that the tested cell lines were in the presence of (**8**) more resistant to membrane damage and cell death. From the above results appeared that the toxicity corresponded to the exposition of aromatic linker included in tested compounds. The reason for **5b** toxicity shown in the Table 1 is not fully understood.

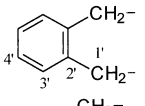


4a X = $-CH_2-$

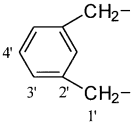
4b X = $-(CH_2)_6-$



4c X =



4d X =



4e X =

NK cell-mediated cytotoxic activity in vitro

The effect of terguride derivatives was tested also for the modulation of immune responses, represented by NK cell-mediated cytotoxicity. On the basis of demonstrated cellular toxicity of high concentrations of terguride dimers (**4c–e**) for the lymphoid cell lines, we used lower concentrations ranging from 10^{-8} to 10^{-10} M. The activity of NK cells was measured in vitro by determining their ability to lyse radiolabeled YAC-1 (NK-sensitive) target cells. The amount of radioactivity released in cell supernatant represents the amount of tumors lysed by the NK cells. Effector cells were incubated 18 h in the presence of terguride clusters or parent compound prior to the addition of ^{51}Cr-labeled target cells. As a reference value (control), the splenocytes were incubated with complete medium RPMI 1640 (see Methods) instead of substances. All assays were performed at effector to target (E/T) ratio 64:1. Results in Tables 2a–c are expressed for better interpretation in the% of controls (untreated lymphocytes). Terguride used in vitro increased the NK cell cytotoxicity in 10–30%. The terguride dimers with aliphatic spacers (**4a–b**) exhibit similar activity as the parent compound (Table 2a), with the exception of (**5b**), which was inhibitory at 10^{-8} M, possibly due to its direct toxicity as shown in the Table 1. The terguride dimers with aromatic linkers (**4c–e**) suppress the NK cell function at the concentration of 10^{-8} M (Table 2b). When added in 10^{-9}–10^{-10} M, the lytic activity of NK cells was not influenced. Table 2c demonstrates the results obtained with aromatic terguride oligomers (**6–8**). They had similar or stronger stimulatory activity than the parent compound. In the case of (**5c**), we found a stable enhancement of NK activity in all tested concentrations. The stimulatory action of (**5c**) probably combines the positive effect of aliphatic spacer with the oligomeric effect.

NK cell-mediated cytotoxic activity ex vivo

Balb/c mice (six animals per group) were injected intraperitonealy by 4 doses (as indicated in Table 3) of terguride or derivatives ones a week. On day 7 after the last treatment, NK cell-mediated cytotoxicity of spleen mononuclear cells was tested against YAC-1 targets. For ex vivo experiments, only one representative of terguride oligomers from three groups—aliphatic linker (**4a**), aromatic linker (**4c**), oligomers (**7**, **8**)—and the parental compound (**2**) were chosen. The results presented in Table 3 show the average value of spleen NK cell activity of all mice in groups. The immunosuppressive effect of low dose terguride (10^{-8}, 10^{-9}) in comparison to the stimulation in vitro may be related either to the repeated doses (chronic treatment well known also from our previous in vitro experiments),[10] or to the involvement of neuroendocrine regulation in vivo, and subsequent downmodulation of NK cell effector function. According to the results shown in Table 3, the effect of terguride oligomers is dose-dependent, where 10^{-7} M is stimulatory for (**4c** and **7**) and the dose lowering 10^{-8}, 10^{-9} M triggers the inhibition of cytotoxicity. Only the terguride hexamer (**8**) kept stimulatory effect in all tested doses used for in vivo treatment.

Conclusions

One of the most important finding of our work was high cellular toxicity and also immunosuppressive activity (at 10^{-8} M concn) exhibited by the terguride dimers with aromatic linker (**4c**–**e**). The toxicity level is directly related to the exposition of aromatic nucleus of the linker (**4c** > **4e** > **4d**). No cellular toxicity on lymphomas of both **5c** and **6** were detected. However, NK cell-mediated cytolytic activity was increased in **5c** (aliphatic linker) and was not influenced by **6** (aromatic core). The beneficial effects (cytoprotective and NK cell stimulatory) appear in tetrameric (**7**) or hexameric (**8**) oligomers of terguride. Terguride hexamer (**8**) is able to protect the cells against spontaneous death (apoptosis) and shows both in vitro and in vivo strong immunostimulatory activity.

It could be concluded that higher oligomerization of terguride transforms its immunosuppressive effect to the immunostimulatory one. The compounds (**5c**) and (**8**) could be the best candidates for further studies. More detailed experiments should be carried out to elucidate the pharmacodynamics and neuroendocrine properties and mechanism of action of new terguride oligomers.

Experimental

Spectroscopy

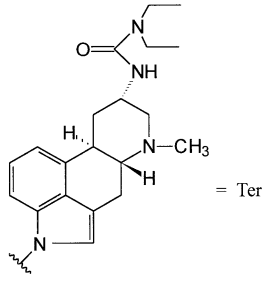
NMR spectra were measured on a Varian INOVA-400 spectrometer (399.90 and 100.57 MHz for ^{1}H and ^{13}C, respectively) in $CDCl_3$ (when not otherwise stated) at 30 °C. Chemical shifts were referenced to the residual solvent signal (δ_H 7.265, δ_C 77.0). 2D NMR experiments (gCOSY, TOCSY, ROESY, HOM2DJ, HMQC, HMBC) were run using the default parameters in the available Varian software. The pulse sequence for 1D-TOCSY was obtained from the Varian User Library.

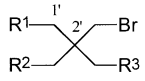
Positive-ion electrospray ionization (ESI) mass spectra were recorded on a double-focusing instrument Finnigan MAT 95 (Finnigan MAT, Bremen, Germany) with BE geometry. Samples dissolved in methanol/water (2:1, v/v) were continuously infused through a stainless capillary held at 3.8 kV into Finnigan ESI source via linear syringe pump at a flow rate of 60 µl/min. A mixture of polypropylene glycols (average $M_r = 725$) was used as an internal standard. All investigated compounds exhibited intense $[M+H]^+$ peaks in ESI MS.

Positive ion MALDI mass spectra were measured on a Bruker Biflex reflectron time-of-flight mass spectrometer (Bruker-Franzen, Bremen, Germany) equipped with a multiprobe sample inlet, a griddles delayed extraction ion source and a nitrogen laser (337 nm). A saturated solution of α-cyano-4-hydroxycinnamic acid in aqueous 50% acetonitrile/0.1% TFA was used as a MALDI matrix. Spectra were calibrated externally using the monoisotopic $[M+H]^+$ ion of α-cyano-4-hydroxycinnamic acid and a peptide standard (angiotensin II, Aldrich).

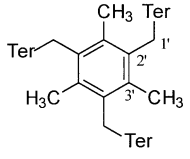
Experimental animals and cell preparations

Inbred female Balb/c mice (Anlab, Germany) at 3 months of age were used for ex vivo experiments. Animals were bleeded and spleens removed. To obtain mononuclear cells, mouse spleens were homogenized in





5a R^1 = Ter, $R^2 = R^3$ = Br
5b $R^1 = R^2$ = Ter, R^3 = Br
5c $R^1 = R^2 = R^3$ = Ter



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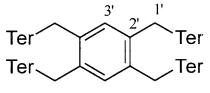

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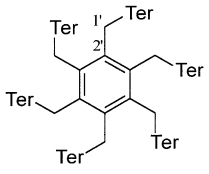

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glass Elwehjem–Potter homogenizer and separated on Ficoll–Hypaque density gradient. After repeated washing the cells were used immediately for in vitro assays.

Established cell lines were maintained in humidified atmosphere with 5% CO_2 at 37 °C in the complete cultivation medium RPMI 1640, supplemented with 0.05 mg/mL gentamycin, 2 mM L-glutamine, 1 mM sodium pyruvate, 0.05 mM 2-mercaptoethanol and 10% heat-inactivated foetal calf serum (FCS) (Gibco, Grand Island, NY, USA). The direct cell toxicity and NK cell activity assays were performed in RPMI 1640 medium supplemented with L-glutamine, gentamycin and 3% of FCS only.

Direct toxicity assay on tumor cells

The effect of TRG derivatives on tumor cells was evaluated using ^{51}Cr ($Na_2{}^{51}CrO_4$) labeled YAC-1 mouse T lymphoma and K562 human erythroleukemic cell lines. ^{51}Cr release was measured after 18 h of incubation in 37 °C humidified CO_2 atmosphere. Cell free supernatants 0.025 mL/sample were harvested and mixed with scintillation cocktail SuperMix (Pharmacia) and measured in β-counter-Microbeta Trilux (Wallac, Finland). Results are calculated as % of spontaneous release from cpm of experimental samples (amount of ^{51}Cr measured in supernatants of cell cultivated in the presence of tested compounds) and control values (spontaneous ^{51}Cr release of cells cultivated without substances).

NK cell-mediated cytolysis assay

Mouse spleen mononuclear cells were used for measuring of in vitro and ex vivo NK cell cytotoxicity. The effector cells were incubated 18 h in the presence of tested substances, before the addition of ^{51}Cr prelabeled (60 min) NK-sensitive YAC-1 tumor targets. Measurement of NK cell activity was performed after additional 18 h of cultivation in CO_2 incubator.

Effector cells at estimated concentrations were incubated with 10^4 target cells/well in 96-well microtiter plates (NUNC). After 18 h of incubation, the supernatants were harvested and radioactivity measured in 0.025 mL as above. The details of the biological methods were published elsewhere.[3–5]

Table 3. The effect of terguride oligomers on NK cell activity measured ex vivo[a]

Compound	Concentration (M)		
	10^{-7}	10^{-8}	10^{-9}
None (control)	100	100	100
2	93	85	75
4c	97	71	117
4e	140	79	71
7	158	78	53
8	109	115	142

[a]The indicated doses were applied in vivo to Balb/c mice (30 g body weight) once a week (four times). Cytotoxicity of spleen mononuclear cells was measured in standard 18 h ^{51}Cr-release assay against YAC-1 target cells in E/T ratio 64:1. Results are expressed in the % of control (effector/target cell mixture incubated in the absence of tested substances = 31% of specific cytotoxicity −100%).

Typical procedure for alkaloid dimer synthesis

Finely powdered KOH (370 mg, 6.6 mmol) was stirred with DMSO (1 mL) for 10 min. Respective alkaloid (0.5 mmol) was added and the stirring was continued for another 30 min. The mixture turns green, which indicates protonization of the indolic N–H. The mixture was cooled to 10–15 °C and the respective α,ω-dihalogen spacer (0.26 mmol, dissolved in 0.1–0.5 mL DMSO) was added in portions. Stirring was continued at the room temperature till the reaction is completed (typically 1.5–2 h) as indicated by TLC: silica gel F_{254} plates (Merck), solvent system $CHCl_3$/MeOH 85:15 (v/v). The spots were visualized by UV light and by charring with 10% H_2SO_4 in ethanol where unsubstituted alkaloids give blue spots and N-1-substiuted alkaloids (dimers) give gray spots. Reaction mixture was poured into the water (20 mL), precipitate of the product was filtered of and washed with water till neutral pH, dried and dissolved in chloroform. The product was either crystallized or purified by a flash chromatography.

Di(8,9-didehydro-6,8-dimethylergoline-1-yl)methane (3a). Typical procedure was used. Dichloromethane (1.5 mmol, 0.1 mL) served as a spacer. Reaction was slow and therefore it was continued overnight (16 h). The precipitate of the product was dissolved in chloroform (4 mL) and MeOH (3 mL) was added. **3a** crystallized after cooling (overnight) affording white fine crystals that were recrystallized from $CHCl_3$/MeOH 1:1 (v/v) to yield 48 mg (40%) of pure **3a**. For NMR, see Tables 4 and 5, MS MALDI TOF $[M+H]^+$ (found 489.35, required 489.30 for $C_{33}H_{37}N_4$). Anal. calcd for $C_{33}H_{36}N_4$: C, 81.1; H, 7.4; N, 11.5; found C, 80.9; H, 7.5; N, 11.6.

1,4-Di(8,9-didehydro-6,8-dimethylergoline-1-yl-methyl)benzene (3b). *p*-Bis(bromo-methyl)benzene[20] (0.25 mmol, 66 mg) was used as a spacer in the typical procedure. The reaction was terminated after 1.5 h. Green pre-

Table 4. ^1H NMR chemical shifts (399.90 MHz, $CDCl_3$, 30 °C) of agroclavine dimers

Proton	**1**[a]	**3a**[b]	**3b**[b]
2	6.899	6.853	6.757
4e	3.320	3.255	3.300
4a	2.773	2.735	2.785
5	2.513	2.523	2.553
7e	2.920	2.959	2.954
7a	3.242	3.250	3.259
9	6.169	6.147	6.177
10	3.738	3.710	3.751
12	6.976	7.022	6.989
13	7.154	7.214	7.140
14	7.154	7.253	7.029
17	1.769	1.769	1.786
N–Me	2.491	2.482	2.502
N–H	8.059	—	—

[a]Ref 22.
[b]Additional signals, spacer protons: **3a**: 6.239 (1H, s); **3b**: 5.207 (2H, s, CH_2), 7.039 (2H, s).

cipitate was after washing and drying purified by flash chromatography (silica gel, $CHCl_3$–MeOH–NH_4OH_{aq} 92:8:0.1) affording green amorphous **3b** (123 mg, 85%). For NMR, see Tables 4 and 5, MS ESI $[M+H]^+$ (found 579.4, required 579.35 for $C_{40}H_{43}N_4$). Anal. calcd for $C_{40}H_{42}N_4$: C, 83.0; H, 7.3; N, 9.7; found C, 83.0; H, 7.4; N, 9.6.

Di(6-methyl-8α-(diethylcarbamoylamino)ergoline-1-yl)-methane (4a). A typical procedure was used. CH_2Cl_2 (1.5 mmol, 0.1 mL) served as a spacer. Reaction was slower and it was terminated after 8 h. The precipitate was recrystallized from Me_2CO/EtOAc 1:1 affording fine yellowish crystals of **4a** (133 mg, 77%). For NMR data see Tables 6 and 7, MS ESI $[M+H]^+$ (found 693.5, required 693.460 for $C_{41}H_{57}N_8O_2$). Anal. calcd for $C_{41}H_{56}N_8O_2$: C, 71.1; H, 8.1; N, 16.1; O, 4.7; found C, 70.9; H, 8.2; N, 15.9; O, 5.0.

1,6-Di(6-methyl-8α-(diethylcarbamoylamino)ergoline-1-yl)-hexane (4b). 1,6-Dibromo-hexane (Aldrich) (0.26 mmol, 0.04 mL) was used as a spacer in the typical procedure. The reaction was quenched after 1.5 h by pouring the mixture into water (20 mL). The precipitate was recrystallized from Me_2CO/EtOAc 9:1 affording fine white crystals of **4b** (109 mg, 57%). For NMR data, see Tables 6 and 7, MS ESI $[M+H]^+$ (found 763.7, required 763.54 for $C_{46}H_{67}N_8O_2$). Anal. calcd for $C_{46}H_{66}N_8O_2$: C, 72.4; H, 8.7; N, 14.7; O, 4.2; found C, 72.1; H, 8.8; N, 14.6; O, 4.5.

1,4-Di(6-methyl-8α-(diethylcarbamoylamino)ergoline-1-yl-methyl)benzene (4c). *p*-Bis(bromomethyl)benzene[20] (0.25 mmol, 66 mg) was used as a spacer in the typical procedure. The reaction was terminated after 1.5 h. The precipitate was recrystallized from Me_2CO/EtOAc 11:1 affording fine yellow crystals of **4c** (122 mg, 62%). For NMR data, see Tables 6 and 7, MS ESI $[M+H]^+$ (found 783.6, required 783.51 for $C_{48}H_{63}N_8O_2$). Anal. calcd for $C_{48}H_{62}N_8O_2$: C, 73.6; H, 8.0; N, 14.3; O, 4.1; found C, 73.4; H, 7.8; N, 14.5; O, 4.3.

Table 5. ^{13}C NMR chemical shifts (399.90 MHz, $CDCl_3$, 30 °C) of agroclavine dimers

Carbon	**1**[a]	**3a**[b]	**3b**[b]
2	117.85	120.88	121.81
3	112.16	112.97	111.97
4	26.69	26.23	26.66
5	63.85	63.49	63.92
7	60.63	60.17	60.71
8	132.25	133.91	132.26
9	119.41	119.39	119.45
10	40.95	40.21	40.89
11	132.42	132.65	132.82
12	112.62	113.53	112.59
13	122.84	123.51	122.81
14	108.51	107.00	107.17
15	133.54	131.74	134.32
16	116.33	127.16	126.96
17	20.83	20.75	20.81
N-Me	40.85	40.21	40.83

[a]Ref 22.
[b]Additional signals, spacer carbons: **3a**: 56.40 t (C-1′); **3b**: 49.82 t (C-1′), 127.24 d (C-3′), 137.38 (C-2′).

1,2-Di(6-methyl-8α-(diethylcarbamoylamino)ergoline-1-yl-methyl)benzene (4d). *o*-Bis(bromomethyl)benzene[20] (0.25 mmol, 66 mg) was used as a spacer in the typical procedure. The reaction was terminated after 1.5 h. The precipitate was recrystallized from pure Me_2CO affording fine yellow crystals of **4d** (113 mg, 58%). For NMR data, see Tables 6 and 7, MS ESI $[M+H]^+$ (found 783.6, required 783.51 for $C_{48}H_{63}N_8O_2$). Anal. calcd for $C_{48}H_{62}N_8O_2$: C, 73.6; H, 8.0; N, 14.3; O, 4.1; found C, 73.5; H, 7.9; N, 14.4; O, 4.2.

1,3-Di(6-methyl-8α-(diethylcarbamoylamino)ergoline-1-yl-methyl)benzene (4e). *m*-Bis(bromomethyl)benzene[20] (0.25 mmol, 66 mg) was used as a spacer in the typical procedure. The reaction was terminated after 1.5 h. The precipitate was recrystallized from Me_2CO affording fine yellowish crystals of **4d** (131 mg, 67%). For NMR data, see Tables 6 and 7, MS ESI $[M+H]^+$ (found 783.6, required 783.51 for $C_{48}H_{63}N_8O_2$). Anal. calcd for $C_{48}H_{62}N_8O_2$: C, 73.6; H, 8.0; N, 14.3; O, 4.1; found C, 73.4; H, 7.8; N, 14.5; O, 4.3.

Terguride oligomers based on the tetrabromopentaerythritol core. Finely powdered KOH (370 mg, 6.6 mmol) was stirred with DMSO (1 mL) for 10 min. Compound **2** (170 mg, 0.5 mmol) was added and the stirring was continued for another 30 min. The mixture was cooled to 10–15 °C and tetrabromopentaerythritol (Aldrich) (49 mg, 0.125 mmol, dissolved in 0.7 mL DMSO) was added during 30 min. Stirring was continued at room temperature for 20 h). TLC ($CHCl_3$/MeOH 93:7) has shown formation of three new products. Reaction mixture was poured in the water (20 mL) and the precipitate was after washing (H_2O) and drying separated by flash chromatography (silica gel, $CHCl_3$/MeOH/NH_4OH_{aq} 93:7:0.1). Three products were isolated and recrystallized from Me_2CO/EtOAc 6:5: **1,3-dibromo-2-bromomethyl-2-(6-methyl-8α-(diethylcarbamoylamino)ergoline-1-yl-methyl)propane (5a)**, R_f 0.71 (5 mg, 6%), MS ESI $[M+H]^+$ (found 645.1, required 645.04 for $C_{25}H_{36}Br_3N_4O$); anal. calcd for $C_{25}H_{35}Br_3N_4O$: C, 46.4; H, 5.5; Br 37.0; N, 8.7; O, 2.4; found C, 46.6; H, 5.4; Br, 37.3; N, 8.6; O, 2.1; **1,3-dibromo-2,2-di(6-methyl-8α-(diethylcarbamoylamino)ergoline-1-yl-methyl)propane (5b)**, R_f 0.54 (52 mg, 46%), MS ESI $[M+H]^+$ (found 905.5, required 905.34 for $C_{45}H_{63}Br_2N_8O_2$); anal. calcd for $C_{45}H_{62}Br_2N_8O_2$: C, 59.6; H, 6.9; Br 17.6; N, 12.4; O, 3.5; found C, 59.8; H, 7.0; Br, 17.6; N, 12.3; O, 3.3; and **1-bromo-2,2-di(6-methyl-8α-(diethylcarbamoylamino)ergoline-1-yl-methyl)-3-(6-methy-8α-(diethylcarbamoylamino)-ergoline-1-yl)propane (5c)**, R_f 0.30 (46 mg, 32%), MS ESI $[M+H]^+$ (found 1165.8, required 1165.64 for $C_{65}H_{90}BrN_{12}O_3$); anal. calcd for $C_{65}H_{89}BrN_{12}O_3$: C, 66.9; H, 7.7; Br 6.9; N, 14.4; O, 4.1; found C, 66.9; H, 7.8; Br, 7.0; N, 14.4; O, 3.9; for NMR data, see Tables 6 and 7.

1,3,5-Tri(6-methyl-8α-(diethylcarbamoylamino)ergoline-1-yl-methyl)-2,4,6-trimethyl benzene (6). Compound **2** (170 mg, 0.5 mmol) and 1,3,5-trimethyl-2,4,6-tris(-bromomethyl)benzene[20] (0.167 mmol, 66.5 mg) were reacted as in the typical procedure for 3 h. The precipitate was recrystallized from EtOH/H_2O 3:1 afford-

ing fine yellowish crystals of **6** (123 mg, 63%). For NMR data, see Tables 6 and 7, MS ESI $[M+H]^+$ (found 1177.9, required 1177.78 for $C_{72}H_{97}N_{12}O_3$). Anal. calcd for $C_{72}H_{96}N_{12}O_3$: C, 73.4; H, 8.2; N, 14.3; O, 4.1; found C, 73.5; H, 8.1; N, 14.4; O, 4.0.

1,2,4,5-Tetra(6-methyl-8α-(diethylcarbamoylamino)ergoline-1-yl-methyl)benzene (7). Compound **2** (170 mg, 0.5 mmol) and 1,2,4,5-tetrakis(bromomethyl)benzene (Aldrich) (0.125 mmol, 56 mg) were reacted as in the typical procedure for 2 h. The precipitate was recrystallized from EtOH/H_2O 4:1 affording (after longer standing in the refrigerator) white microcrystals of **7** (77 mg, 41%). For NMR data, see Tables 6 and 7, MS ESI $[M+H]^+$ (found 1487.9, required 1487.97 for $C_{90}H_{119}N_{16}O_4$). Anal. calcd for $C_{90}H_{118}N_{16}O_4$: C, 72.6; H, 8.0; N, 15.1; O, 4.3; found C, 72.5; H, 7.9; N, 15.4; O, 4.2.

Table 6. ^{1}H NMR chemical shifts (399.90 MHz, $CDCl_3$, 30 °C) of terguride dimers and oligomers

Proton	2[a]	4a	4b	4c	4d	4e	5a	5b	5c	6	7	8
2	6.895	6.850	6.711	6.747	6.622	6.747	7.056	7.041	6.813	6.427	6.489	6.393
4*e*	3.414	3.300	3.356	3.351	3.352	3.370	3.380	3.398	3.385	3.335	3.307	3.288
4*a*	2.698	2.577	2.650	2.645	2.655	2.666	2.643	2.663	2.668	2.614	2.610	2.614
5	2.211	2.128	2.189	2.194	2.207	2.215	2.183	2.196	2.191	2.187	2.162	2.178
7*e*	2.890	2.773	2.867	2.867	2.873	2.877	2.877	2.877	2.884	2.868	2.794	2.889
7*a*	2.484	2.447	2.475	2.476	2.486	2.490	2.486	2.491	2.493	2.473	2.476	2.491
8	4.315	4.253	4.273	4.273	4.278	4.282	4.274	4.275	4.278	4.275	4.270	4.274
9*e*	2.813	2.842	2.832	2.790	2.800	2.807	2.802	2.791	2.790	2.802	2.868	2.796
9*a*	1.634	1.590	1.628	1.627	1.637	1.643	1.627	1.625	1.620	1.631	1.613	1.613
10	3.088	2.986	3.035	3.040	3.057	3.061	3.028	3.036	3.042	3.041	3.032	3.032
12	6.857	6.913	6.887	6.878	6.892	6.898	6.925	6.889	6.876	6.914	6.883	6.651
13	7.135	7.196	7.139	7.116	7.103	7.129	7.201	7.124	7.087	7.172	7.064	7.027
14	7.182	7.374	7.061	7.025	6.909	7.015	7.349	6.978	6.740	7.117	6.757	6.911
NMe	2.417	2.371	2.411	2.403	2.411	2.418	2.422	2.430	2.432	2.396	2.406	2.432
NH	5.645	5.476	5.329	5.528	5.526	5.539	5.491	5.501	5.505	5.498	5.543	5.553
CH_2	3.362	3.325	3.336	3.340	3.339	3.342	3.331	3.335	3.327	3.336	3.338	3.336
	3.266	3.235	3.247	3.250	3.258	3.257	3.260	3.256	3.270	3.246	3.252	3.251
CH_3	1.138	1.138	1.148	1.151	1.157	1.155	1.155	1.156	1.158	1.148	1.150	1.146

Additional signals, spacer protons: terguride (**2**): 8.726 (1H, d, $J=1.8$ Hz, indole NH); **4a**: 6.239 (2H, s, 2×H-1′); **4b**: 1.319 (2H, m, 2×H-2′), 1.782 (2H, m, 2×H-3′), 4.015 (2 H, t, $J=7.0$ Hz, 2×H-1′); **4c**: 5.209 (4H, s, 4×H-1′), 7.046 (2H, s, H-3′, H-4′); **4d**: 5.177 (2H, s, 2×H-1′), 6.961 and 7.228 (4H, AA′BB′, H-3′, H-4′, H-5′, H-6′); **4e**: 5.194 (2H, s, 2×H-1′), 6.991 (2H, dd, $J=7.5$, 1.7 Hz, H-5′, H-7′), 7.034 (1H, d, $J=1.7$ Hz, H-3′), 7.193 (1H, t, $J=7.5$ Hz, H-6′); **5a**: 3.570 (6H, s, 3×CH_2Br), 4.341 (1H, d, $J=15.0$ Hz, H-1′u), 4.362 (1H, d, $J=15.0$ Hz, H-1′d); **5b**: 4.346 (1H, d, $J=15.2$ Hz, H-1′u), 4.387 (1H, d, $J=15.2$ Hz, H-1′d), 3.552 (1H, d, $J=11.2$ Hz, H-3′u), 3.588 (1H, d, $J=11.2$ Hz, H-3′d); **5c**: 3.363 (2H, s, CH_2Br); **6**: 2.306 (3H, =C–CH_3), 5.315 (2H, s, 2×H-1′); **7**: 5.043 (4H, s, 4×H-1′), 6.784 (2H, s, 2×H-3′); **8**: 5.307 (1H, d, $J=15.1$ Hz), 5.311 (1H, d, $J=15.1$ Hz).
[a]Ref 23.

Table 7. ^{13}C NMR chemical shifts (399.90 MHz, $CDCl_3$, 30 °C) of terguride dimers and oligomers

Carbon	2[a]	4a	4b	4c	4d	4e	5a[b]	5b	5c	6	7	8
2	117.86	120.65	121.33	121.66	121.45	121.69	122.0	122.09	121.67	119.60	121.33	119.77
3	111.27	112.77	110.56	111.36	111.62	111.39	112.4	112.76	112.93	111.00	111.72	112.47
4	26.95	26.89	27.01	26.99	27.03	27.03	27.0	27.00	27.00	27.05	26.97	26.88
5	67.57	67.44	67.69	67.65	67.69	67.67	67.4	67.52	67.68	67.65	67.68	67.36
7	61.80	61.95	62.00	61.98	62.00	62.01	61.9	61.99	62.00	61.99	62.00	61.90
8	45.03	44.97	45.01	45.01	45.01	45.03	44.9	44.99	44.98	45.00	44.99	44.92
9	32.52	32.53	32.58	32.56	32.57	32.59	32.5	32.53	32.56	32.57	32.59	32.51
10	36.53	36.58	36.64	36.62	36.65	36.66	36.5	36.65	36.67	36.63	36.63	36.51
11	133.04	113.03	133.64	133.71	134.07	133.73	n.d.[c]	134.02	134.09	133.79	133.88	134.07
12	112.90	113.87	112.65	113.03	113.28	113.07	113.7	113.61	113.65	113.23	113.41	113.89
13	122.85	123.61	122.64	122.96	123.10	122.99	123.5	123.49	123.60	122.94	123.15	123.40
14	108.59	106.82	106.91	107.13	107.14	107.18	107.2	107.66	106.92	106.89	107.13	107.33
15	133.30	133.56	133.66	133.96	133.82	134.00	134.5	135.25	135.38	133.93	133.92	133.91
16	126.21	127.15	126.66	126.85	126.85	126.88	n.d.	126.52	126.33	126.96	126.90	127.15
NMe	43.30	43.37	43.41	43.40	43.43	43.43	43.4	43.44	43.48	43.49	43.33	43.38
NC=O	156.67	146.61	156.63	156.63	156.64	156.64	n.d.	156.61	156.61	156.62	156.62	156.58
CH_3	13.80	13.88	13.88	13.88	13.90	13.90	14.0	13.91	13.91	13.89	13.90	13.87
CH_2	41.09	41.13	41.12	41.13	41.13	41.13	41.2	41.14	41.15	41.13	41.13	41.09
1′		56.34	46.29	49.82	47.73	50.08	47.2	48.13	48.08	45.07	47.68	138.88
2′			26.60	137.39	135.31	138.52	45.1	46.96	48.74	131.95	135.66	
3′			30.44	127.25	128.53	125.61	36.4	37.70	38.42	139.04	130.22	
4′					128.26	138.52				16.35		
5′						126.21						
6′						129.20						

[a]Ref 23.
[b]HMQC readouts.
[c]n.d. = not determined.

1,2,3,4,5,6-Hexa(6-methyl-8α-(diethylcarbamoylamino)-ergoline-1-yl-methyl)benzene (8). To the stirred solution of **2** (170 mg, 0.5 mmol) in DMSO (1 mL) with powdered KOH (370 mg) hexakis(bromomethyl)benzene[20] (0.083 mmol, 53 mg) was added at room temperature. The spacer slowly dissolves in DMSO allowing thus full saturation of core with the alkaloid moieties. Reaction was continued overnight then it was quenched by pouring into the water (20 mL) and the precipitate was thoroughly washed with water and dried. Flash chromatography (silica gel, $CHCl_3/MeOH/NH_4OH_{aq}$ 93:7:0.1) afforded **8** as amorphous foam (118 mg, 65%). For NMR, see Tables 6 and 7, MS ESI $[M+2H]^{2+}$ (found 1097.3, required 1097.71 for $C_{132}H_{176}N_{24}O_6$). Anal. calcd for $C_{132}H_{174}N_{24}O_6$: C, 72.3; H, 8.0; N, 15.3; O, 4.4; found C, 72.2; H, 8.0; N, 15.2; O, 4.6.

1-Allyl-6-methy-8α-(diethylcarbamoylamino)ergoline (9a). To the stirred solution of **2** (510 mg, 1.5 mmol) in DMSO (3 mL) with powdered KOH (1100 mg, 20 mmol) freshly distilled allyl bromide (Merck) (0.175 mL, 2 mmol) was slowly added at room temperature. After 1 h reaction, the mixture was poured into water (50 mL) and the precipitate was after washing and drying recrystallized from EtOAc/hexane 4:1 to afford white crystals of **9a** (492 mg, 86%). For ^1H and ^{13}C NMR, see Tables 8 and 9, respectively. MS ESI $[M+H]^+$ (found 381.3, required 381.27 for $C_{23}H_{33}N_4O$). Anal. calcd for $C_{23}H_{32}N_4O$: C, 72.6; H, 8.5; N, 14.7; O, 4.2; found C, 72.7; H, 8.3; N, 14.6; O, 4.4.

1-Carboxymethyl-6-methy-8α-(diethylcarbamoylamino)-ergoline (9b). To the stirred solution of **2** (510 mg, 1.5 mmol) in DMSO (3 mL) with powdered KOH (1100 mg, 20 mmol) ethylbromoacetate (Merck) (0.217 mL, 2 mmol) was slowly added at room temperature. After 1.5 h reaction mixture was poured into water (50 mL) and stirred for 30 min to achieve complete ester hydrolysis. The resulting solution was slowly passed through a column (4×20 cm) of Amberlite XAD-2 previously washed with acetone and MeOH, and equilibrated with water. Then the column was washed with water till neutral reaction and alkaloid derivatives were eluted with MeOH. Solution was concentrated to 10%, dissolved in water (20–30 mL) and acidified with HCl. The precipitate was filtered off, washed, dried and recrystallized from MeOH to afford **9b** (yellowish crystals, 501 mg, 84%). For ^1H and ^{13}C NMR see Tables 8 and 9, respectively. MS ESI $[M+H]^+$ (found 399.3, required 399.24 for $C_{22}H_{31}N_4O_3$). Anal. calcd for $C_{22}H_{30}N_4O_3$: C, 72.6; H, 8.0; N, 15.1; O, 4.3; found C, 72.5; H, 7.9; N, 15.4; O, 4.2.

1-(6-Bromohexyl)-6-methyl-8α-(diethylcarbamoylamino)-ergoline (9c). To the well stirred solution of **2** (681 mg, 2 mmol) in DMSO (4 mL) with powdered KOH (1450 mg, 26 mmol) 1,6-dibromohexane (Aldrich) (1.82 mL, 12 mmol) was quickly added at room temperature. After 1 h, the reaction was quenched with water (50 mL) and the mixture was 3× extracted with $CHCl_3$ (20 mL). The extract was dried and evaporated to a thin syrup. The syrup was triturated 3× with 20 mL of petrolether (to remove bulk of unreacted 1,6-dibromohexane) and then 1× with 20 mL of water (to remove the rest of DMSO). Flash chromatography (silica gel, $CHCl_3/MeOH/NH_4OH_{aq}$ 97.5:2.5:0.05) afforded as a side product **4b** (220 mg, 21%) and **9** (449 mg, 45%). For ^1H and ^{13}C NMR, see Tables 8 and 9, respectively. MS ESI $[M+H]^+$ (found 503.3, required 503.24 for $C_{26}H_{40}BrN_4O$). Anal. calcd for $C_{26}H_{39}BrN_4O$: C, 62.0; H, 7.8; Br, 15.9, N, 11.1; O, 3.2; found C, 61.7; H, 7.9; Br, 16.0; N, 10.9; O, 3.5.

Table 8. ^1H NMR chemical shifts (399.90 MHz, $CDCl_3$, 30 °C) of terguride derivatives with activated spacers

Proton	9a	9b[a]	9c	9d[b]	9e
2	6.773	7.002	6.763	6.872	6.879
4*e*	3.376	3.651	3.373	3.322	3.291
4*a*	2.669	3.133	2.660	2.882	2.557
5	2.213	3.324	2.199	2.494	2.120
7*e*	2.876	3.782	2.868	3.140	2.800
7*a*	2.488	3.476	2.481	2.799	2.375
8	4.281	4.360	4.371	4.302	4.180
9*e*	2.801	2.874	2.779	2.656	2.700
9*a*	1.639	1.930	1.636	1.452	1.492
10	3.053	3.613	3.041	3.268	2.973
12	6.885	6.945	6.875	6.773	6.850
13	7.156	7.191	7.156	7.064	7.156
14	7.099	7.175	7.103	7.170	7.247
NMe	2.421	3.044	2.417	2.587	2.360
NH	5.527	—	5.510	6.259	5.584
CH_2	3.524	3.457	3.349	3.282	3.321
	3.342	3.385	3.261	3.235	3.230
CH_3	1.151	1.181	1.152	1.075	1.128

[a]In CD_3OD'.
[b]$CDCl_3$ + one drop of CD_3OD'. Additional signals, spacer protons: **9a**: 4.675 (2H, dt, J = 5.5,1.6 Hz, 2×H-1′), 5.126 (1H, dddd, J = 17.0, 1.6, 1.6, 1.6 Hz, H-3′$_{trans}$), 5.188 (1H, dddd, J = 10.2, 1.6, 1.5, 1.5 Hz, H-3′$_{cis}$), 5.991 (1H, ddt, J = 17.0, 10.2, 5.5 Hz, H-2′); **9b**: 4.693 (2H, s, NCH_2); **9c**: 1.349 (2H, m, H-3′), 1.469 (2H, m, H-4′), 1.817 (2H, m, H-5′), 1.852 (2H, m, H-2′), 3.376 (2H, m, H-6′), 4.070 (2H, m, H-1′); **9d**: 3.185 (2H, t, J = 6.6 Hz, H-2′), 4.284 (2H, t, J = 6.6 Hz, H-1′); **9e**: 5.560 (2H, s, H-1′).

Table 9. ^{13}C NMR chemical shifts (399.90 MHz, $CDCl_3$, 30 °C) of terguride derivatives with activated spacers[a,b]

Carbon	9a	9b[a]	9c	9d[b]	9e
2	121.46	124.58	121.36	122.22	120.99
3	111.00	109.12	110.64	109.24	112.01
4	26.94	25.65	27.03	25.11	26.71
5	67.79	68.96	67.71	67.13	67.53
7	61.91	60.75	62.02	60.50	61.76
8	44.93	46.72	45.02	44.38	44.92
9	32.55	33.09	32.56	32.06	32.51
10	36.55	36.07	36.67	34.84	36.39
11	133.81	136.32	133.67	133.55	133.45
12	112.97	114.89	112.67	113.44	113.78
13	122.86	125.16	122.68	123.16	123.30
14	107.26	109.31	106.92	107.42	107.24
15	133.60	131.05	133.67	131.38	133.45
16	126.76	127.61	126.69	126.32	127.24
NMe	43.34	42.10	43.42	42.12	43.25
NC=O	156.64	159.62	156.62	156.85	156.75
CH_2[c]	41.14	42.62	41.13	41.05	41.12
CH_3[c]	13.90	14.31	13.90	13.66	13.82

[a]In CD_3OD.
[b]$CDCl_3$ + one drop of CD_3OD.
[c]2 C. Additional signals, spacers: **9a**: 48.99 (C-1′), 117.09 (C-3′), 133.89 (C-2′); **9b**: 48.79 (C-1′), 172.42 (COOH); **9c**: 26.14 (C-3′), 27.76 (C-4′), 30.44 (C-5′), 32.56 (C-2′), 33.59 (C-6′), 46.28 (C-1′); **9d**: 40.25 (C-2′), 45.16 (C-1′); **9e**: 69.76 (C-1′).

1-(2-Aminoethyl)-6-methyl-8α-(diethylcarbamoylamino)-ergoline (9d). To the stirred solution of **2** (510 mg, 1.5 mmol) in DMSO (3 mL) with powdered KOH (1100 mg, 20 mmol) 2-bromoethylamine hydrobromide (Aldrich) (922 mg, 4.5 mmol) dissolved in 3 mL DMSO was dropwise added within 1.5 h at 5–10 °C. After another 1 h stirring at room temperature, the reaction mixture was poured into water (50 mL) and the precipitate is washed till neutral reaction with water and dried. Flash chromatography (silica gel, $CHCl_3$/MeOH/NH_4OH_{aq} 75:25:0.1) afforded **9d** as amorphous foam (351 mg, 61%). ^{1}H NMR (399.90 MHz, $CDCl_3$ + one drop of CD_3OD, 30 °C): For ^{1}H and ^{13}C NMR, see Tables 8 and 9, respectively. MS ESI $[M+H]^+$ (found 384.3, required 384.27 for $C_{22}H_{34}N_5O$). Anal. calcd for $C_{22}H_{33}N_5O$: C, 68.9; H, 8.7; N, 18.3; O, 4.1; found C, 68.7; H, 8.9; N, 17.9; O, 4.5.

1-Hydroxymethyl-6-methyl-8α-(diethylcarbamoylamino)-ergoline (9e). Compound **2** (340 mg, 1 mmol) was refluxed for 1 hour with aqueous formaldehyde (37%, 10 mL). After cooling the mixture was extracted with ethyl acetate (3×10 mL), combined extracts were washed with water and brine. Crude yellowish product (405 mg) was obtained after evaporation. Flash chromatography (silica gel, $CHCl_3$/MeOH/NH_4OH_{aq} 75:25:0.1) afforded **9e** as amorphous foam (351 mg, 61%) **9e** (240 mg, 65%). For ^{1}H and ^{13}C NMR see Tables 8 and 9, respectively. MS ESI $[M+H]^+$ (found 371.3, required 371.24 for $C_{21}H_{31}N_4O_2$). Anal. calcd for $C_{21}H_{30}N_4O_2$: C, 68.1; H, 8.2; N, 15.1; O, 8.6; found C, 68.1; H, 8.3; N, 14.9; O, 8.7.

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