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(N,N-Diethylamino)alkoxy Derivatives of Phenanthrolines as *DNA* Binding Agents

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The interaction of the tricyclic angular azaaromatic system having positive charged side chains with *DNA*, influence of molecular structure of the ligand on the mode of noncovalent binding, and stability of the complex formed were established. Several (N,N-diethylamino)alkoxy derivatives of 1,7-, 1,8-, 1,10- and 4,7-phenanthroline in the protonated form were used as ligand. The electronic and steric factors were shown as responsible for the electrostatic and intercalative interactions of the ligand with *DNA*. The syntheses of (N,N-diethylamino)alkoxy derivatives from parent phenanthrolines were elaborated.

(Keywords: Phenanthroline; Aminoether; Vivakorfen; Intercalation of DNA)

(N,N-Diethylamino)alkoxy-phenanthrolinverbindungen als DNA Bindungsagentien

Die Wechselwirkung von trizyklischen, azaaromatischen Systemen mit positiver Ladung an Seitenketten mit *DNA*, der Einfluß der molekularen Struktur des Liganden bei nonkovalenter Bindung und die Stabilität des gebildeten Komplexes wurde bestimmt. Als Liganden wurden mehrere (N,N-diethylamino)alkoxy-Verbindungen von 1,7-, 1,8-, 1,10- und 4,7-Phenanthrolin in protonierter Form benutzt. Es wurde gezeigt, daß die elektronischen und sterischen Faktoren für elektrostatische und intercalative Wechselwirkungen des Ligand und *DNA* verantwortlich sind. Es wurden Synthesen von (N,N-diethylamino)alkoxy-phenanthrolinen aus entsprechenden Chlorverbindungen ausgearbeitet.

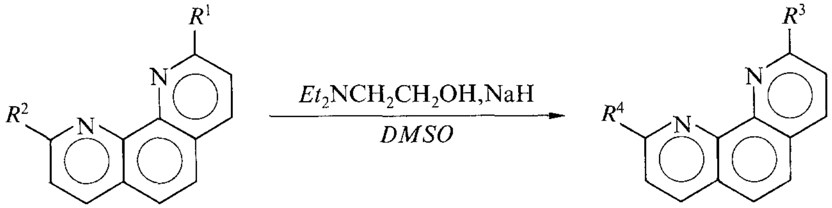
Introduction

In our recent publications [1, 2] we focused our attention on the tricyclic azines with (N,N-dialkylamino)alkoxy substituents as potential novel synthetic interferon inducers noncovalently bound to *DNA*. Among these compounds 3,8-bis[2-(N,N-diethylamino)ethoxy]-4,7-phenanthroline dihydrochloride (**19**), named vivakorfen, was revealed as a ligand strongly interacting with *DNA* and because of its spectroscopic properties it can be considered as a novel *DNA* fluorescent probe [3] similar to the well known ethidium bromide [4].

The main aim of this work was to clear the relations between the structure of angular tricyclic azaaromatic system with variously situated cationic side chains and their ability to noncovalent binding to *DNA*. Thus, syntheses of 1,7-, 1,8-, 1-10- and 4,7-phenanthrolines mono- or disubstituted with the (N,N-diethylamino)ethoxy group were elaborated and the properties of these compounds were established.

Results and Discussion

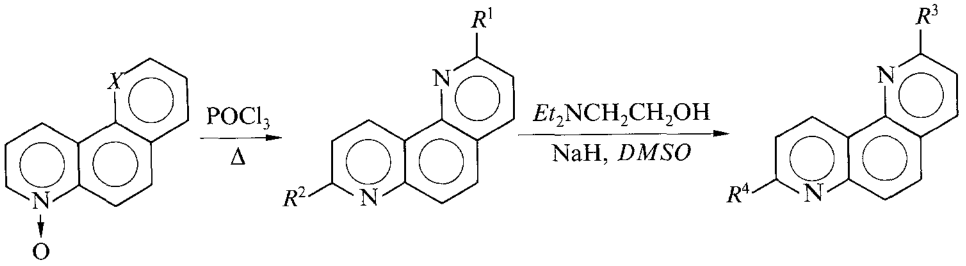
2-[2-(N,N-diethylamino)ethoxy]- and 2,9-bis[2-(N,N-diethyl-amino)ethoxy]-1,10-phenanthrolines (**3** and **4**) were obtained from the corresponding mono- or dichloro-1,10-phenanthroline (**1** and **2**) synthesized according to known procedures [5, 6].



1 $R^1 = H$, $R^2 = Cl$
2 $R^1 = R^2 = Cl$

3 $R^3 = H$, $R^4 = OCH_2CH_2NEt_2$
4 $R^3 = R^4 = OCH_2CH_2NEt_2$

8-[2-(N,N-diethylamino)ethoxy]- and 2,8-bis[2-(N,N-diethylamino)-ethoxy]-1,7-phenanthroline (**9** and **10**) were obtained from the corresponding chloro derivatives **7** and **8** synthesized from known N-oxides **5** and **6** with phosphorus oxychloride [7].

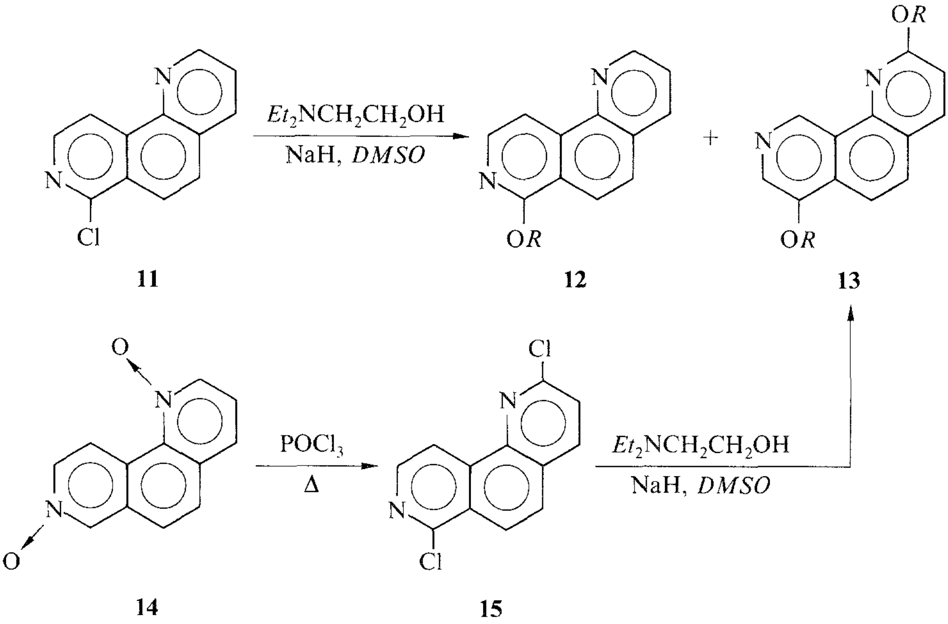


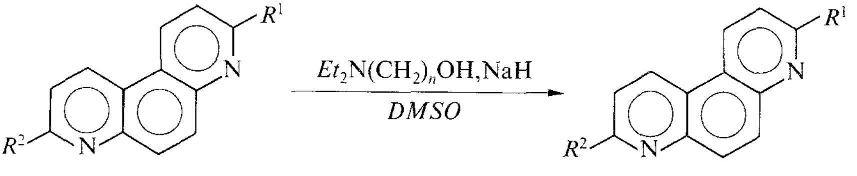
5 $X = N$
6 $X = N \rightarrow O$

7 $R^1 = H$, $R^2 = Cl$
8 $R^1 = R^2 = Cl$

9 $R^3 = H$, $R^4 = OCH_2CH_2NEt_2$
10 $R^3 = R^4 = OCH_2CH_2NEt_2$

Similar reactions were carried out on the 1,8-phenanthroline system. Surprisingly, the chloro derivative **11** gave with 2-(N,N-diethylamino)ethanol a product of substitution of the chlorine atom **12** besides of a substantial amount of diether **13**. Thus, particular activation of the C-2 ring atom towards nucleophile in the 1,8-phenanthroline system took place. The nucleophilic substitution of hydrogen atoms did not occur in unsubstituted 1,8-phenanthroline under the same conditions, even at the most active C-7 position. On the other hand the diether **13** was also easily obtained from 2,7-dichloro-1,8-phenanthroline **15**, prepared from known di-N-oxide **14** [8].





16 $R^1 = Cl$ $R^2 = H$
17 $R^1 = R^2 = Cl$

$n = 2, 3$ or 5

18 $R^1 = OCH_2CH_2NEt_2$, $R^2 = H$
19 $R^1 = R^2 = OCH_2CH_2NEt_2$
20 $R^1 = R^2 = O(CH_2)_3NEt_2$
21 $R^1 = R^2 = O(CH_2)_5NEt_2$

Vivakorfen **19**, in the free base form, and its close analogs **18**–**21** were obtained from 3-chloro- and 3,8-dichloro-4,7-phenanthroline (**16** and **17**) [9] and appropriate N-N-diethylaminoalcohols in the same manner as compounds **3** and **4**.

All phenanthroline aminoethers obtained (**3**, **4**, **9**, **10**, **12**, **13**, **18**–**21**) were transformed into water soluble hydrochlorides having, under measuring conditions ($pH = 7.0$), positive charges localized on the sidechain nitrogen atoms, due to their basicity higher ($pK_a = 9.2$) [10] than the basicity of the heterocyclic nitrogen atoms ($pK_{aI} = 4.0 - 5.2$) [11].

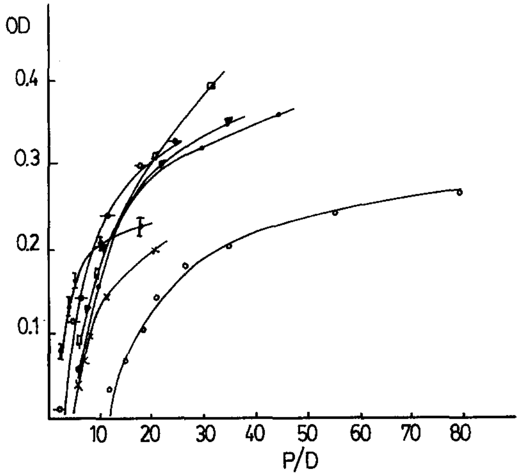


Fig. 1. Hypochromic effects on mixing ligands with CT-*DNA* at a given wavelength at different *P*/*D*. *DNA* concentration $P = 8 \cdot 10^{-5} M$, 5 m*M* *TRIS*/HCl buffer, *pH* 7.0. **3** (▼ 230 nm), **4** (□ 230), **9** (× 232), **10** (● 232), **12** and **13** (⊖ 230), **18** (ɸ) 232) and vivakorfen (○ 238)

These hydrochlorides were then used as ligands for complexes with calf thymus *DNA*. The process of complex formation run at low ionic strength and neutral *pH* and the respective UV spectra were recorded. A hypochromic effect was observed at the main ligand absorption band (Fig. 1), most pronounced at low *DNA*-ligand ratios (*P*/*D*); it was partially reversible with increase of the Na$^+$ concentration up to 0.2 *M*. These facts suggest that some amount of ligand molecules is bound to *DNA* electrostatically at low ionic strength [2].

The stabilization of the *DNA* secondary structure by various ligands was established by the measurement of the melting points of complexes at different Na$^+$ concentrations at $P/D = 5$ (Table 1).

The data obtained for various complexes revealed that both cationic side chains in the ligand molecule are structural elements necessary for high thermal stabilization of *DNA*. The results found seem to confirm the earlier formulated supposition [2] that in low ionic strength dicationic side-chains of ligand interact electrostatically with the phosphate oxygen atoms of both *DNA* strands, thus orienting the aromatic rings system of the ligand perpendicularly to the *DNA* helix axis. Then, intercalation of some of the bound ligand molecules occurred. Electrostatically bound ligand could be released from the complex by titration with counterions (e.g. Na^+, Mg^{2+}). The intercalated ligand molecules probably remained bound to *DNA* even at high ionic strength.

Table 1. *Stability of the DNA secondary structure in complexes with phenanthroline derivatives at different* Na^+ *concentrations. DNA* $\cdot 10^{-5}$ *M*, 5 m*M* *TRIS*/HCl, *pH* 7.0, *P*/*D* – 5.0

Compound	NaCl (m*M*)					ΔT_m (°C) in *TRIS* buffer
	0	3	10	30	100	
19	86.5	85.0	85.0	—	—	20.0
20	82.5	—	81.0	—	—	16.0
21	82.0	—	81.5	—	—	15.5
18	74.0	74.0	76.0	78.0	83.0	7.5
9	71.0	73.0	74.9	78.0	83.0	4.5
10	83.5	82.5	82.0	83.0	83.0	17.0
12	74.0	74.5	74.0	—	—	7.5
13	74.0	74.0	74.5	—	—	7.5
3	74.5	74.5	74.0	78.0	83.0	8.0
4	81.0	81.0	81.0	—	—	14.5
DNA (control)	66.5	69.0	71.0	78.0	83.0	

As a measure of the electrostatic affinity of the ligand to *DNA*, the Na^+ concentration at which half of the electrostatically bound ligand molecules were released from the complex (c_m) was taken. The data found (Table 2) gave the evidence that the compounds having two side chains, formed more stable complexes with *DNA* than other ligands being under investigation, except the dication of **13**.

According to our previous determinations [2], the binding constant for electrostatic interactions of vivakorfen with calf thymus *DNA* was $K_1 = 6.54 \cdot 10^4 M^{-1}$, whereas the isomerisation constant for the intercalation was $K_2 = 33$. This means that at low *P*/*D* and low ionic strength, when most or all *DNA* binding sites are filled up, intercalation could play an important role in *DNA* stabilization.

Table 2. *Fluorescence properties of phenanthroline derivatives (protonated and neutral species) and of their complexes with calf thymus DNA.* Ligands concentration $1 \cdot 10^{-6} M$, 5 m*M TRIS*/HCl, *pH* 7.0

Compound	I_0/I_n	$\Delta\lambda_n$ (nm)[a]	I_0/I_b[b]	$\Delta\lambda_c$ (nm)	I_1/I_b[c]	c_m (*M*)[d]	*P*/*D*[e]
19	32.0	7	21.4	5	18.4	0.14	200
18	8.4	15	3.3	5	2.7	0.0145	116
9	8.2	7	3.3	3	2.7	0.0145	159
10	6.9	10	5.2	14	4.6	0.064	80
12	6.4	7	4.1	5	2.7	0.018	176
13	1.5	0	2.7	0	2.4	0.015	73
3	2.6	0	2.6	0	2.7	0.0085	89
4	4.2	6	4.0	3	4.1	0.021	67

[a] I_0 Fluorescence intensity at the main fluorescence spectrum band of the free, protonated ligand; I_n fluorescence intensity at the above wavelength for neutral free ligand (*pH* 11.5); $\Delta\lambda_n$ batochromic shift of the main fluorescence spectrum band on neutralization

[b] I_b Fluorescence intensity at the main fluorescence spectrum band of a free ligand, after complexing with *DNA*, when essentially no free ligand was present in a solution; $\Delta\lambda_c$ batochromic shift of the main fluorescence spectrum band upon complex formation

[c] I_1 Fluorescence intensity at the above wavelength at the end of Na^+ titration

[d] c_m Concentration of Na^+ at which half of ligand molecules were released from the complex

[e] *P*/*D* The molar ratio of *DNA* (*P*) to ligand (*D*) at the end of complex formation

Table 3. *Electrophoretic evidence for unwinding of supercoiled PM2 DNA by phenanthroline derivatives added to the gel*

Compound	Equivalence point[a] (μq/ml)	
	Monocationic derivative	Dicationic derivative
4,7-Phenanthroline	20	2.5 (**19**), 5.5 (**20**), 5.9 (**21**)
1,7-Phenanthroline	25	6.0
1,10-Phenanthroline	20	10.0
1,8-Phenanthroline	40	12.0

[a] The concentration of ligand in the gel at which supercoiled *DNA* migrates with open circular form

Another experiment, involving measurements of equivalence points for unwinding of supercoiled PM 2 *DNA* by ligands, showed that complete unwinding occurred at lower ligand concentrations for all dicationic derivatives as compared to monocations. This result is in accordance with an intercalative mode of interaction between ligand and *DNA* molecules.

The facts observed and discussed above as well as some data given in the literature [12] suggest that the process of noncovalent interaction of tricyclic azines with cationic side chains with *DNA* could be influenced mainly by two factors—charge distribution in the ligand molecule and steric conditions of the aromatic ring system.

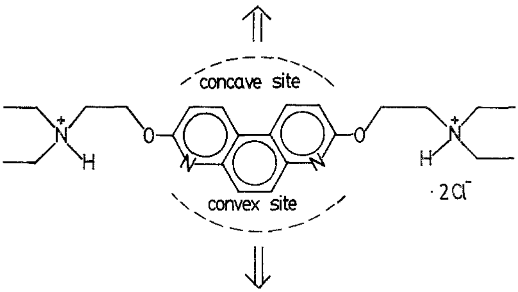


Fig. 2. The mode of insertion of vivakorfen into *DNA*

The crucial requirement for the structure of strongly binding ligand is the presence of positive charges localized in the two side chains. The distances between them, calculated for the extremely stretched conformations according to the data given in the literature [11, 13] (for **4** 13.9, **10** 15.8, **13** 15.6, **19** 15.8, **20** 18.8, and **21** 23.5 Å) seem to have secondary importance. Nevertheless, for the homologs (compounds **19**–**21**) the influence of this factor on the ΔT_m values of the complexes and of the equivalence points for unwinding is manifested.

Another factor responsible for stability of the complex formed seemed to be of steric nature. The ligand molecule immobilized on the *DNA* helix could intercalate the inserting aromatic ring system from the convex site or the concave site, as shown in Fig. 2. The first mode should take place in the case of 1,10-phenanthroline **4** as well as 1,7-phenanthroline **10**, both of them are probable for 4,7-phenanthroline **19**. The steric hindrance of both these sites occurred in disubstituted 1,8-phenanthroline **13** making both these modes of intercalation more difficult and the complex formed is less stable.

Vivakorfen and all its close phenanthroline analogs were found to be inactive as interferon inducers in Balb/c mice and in the bone marrow-derived macrophage cultures.

Acknowledgements

Skillfull technical assistance of Mrs. *Hanna Kozlowska* is appreciated. We thank the Polish Academy of Sciences, Grant CPBR 3.13.6. for financial support of this investigation.

Experimental

Melting points of the synthesized compounds, determined on a *Böetius* apparatus, are uncorrected. ^{1}H-NMR spectra were recorded at 100 MHz on a Tesla BS-567 spectrometer in $CDCl_3$ with *HMDSO* as internal standard. IR spectra were measured on a Perkin-Elmer 621 spectrophotometer in KBr. All biochemical materials and methods will be published separately [2]. Shortly, concentration of stock ligand and *DNA* solution were determined spectrophotometrically by determining and using molar extinction coefficients. UV-spectra and melting profiles were recorded on a Unicam 500 with temperature control. Formation of the complex was observed by measuring UV-spectra on mixing calf thymus *DNA* ($1 \times 10^{-4} M$) with ligand at different *P/D* in 5 m*M* *TRIS*/HCl, *pH* 7.0. Ligand was added to both, control and *DNA*-containing cells. Electrophoresis of PM 2 *DNA* was performed according to *Espejo* and *Lebowitz* [14]. Fluorescence spectra were recorded at room temperature on an Aminco Bowman spectrofluorimeter, and were not corrected. Excitation wavelength was chosen as 290 nm assuming that at this wavelength and the *DNA* concentrations used an inner filter effect is avoided. Fluorescence spectra for protonated ligands were taken in 5 m*M* *TRIS*/HCl, *pH* 7.0, and for neutral forms in water (*pH* 11.5). In titration experiments, to the ligand solution ($1 \cdot 10^{-6}$) in 5 m*M* *TRIS*/HCl, *pH* 7.0, exhibiting fluorescence at the main fluorescence spectrum band of a given intensity (I_0) *DNA* and 4 *M* NaCl were successivelly added, both up to the point when the fluorescence did not change (I_b and I_1, respectively).

All tests for interferonogenic activities were carried out by *A. D. Inglot* and coworkers, Laboratory of Tumor Virology, Institute of Immunology and Experimental Therapy, Polish Academy of Sciences in Wrocław, by the method described earlier [1, 15, 16].

8-Chloro-1,7-phenanthroline (7)

1,7-Phenanthroline (**5**) mono-N-oxide (4.0 g, 20 mmol) (obtained as reported in Ref. [7]) was added to a cooled mixture (0–5 °C) of dry benzene (20 ml) and phosphorus oxychloride (18 ml). The mixture was gently refluxed for 30 min. Then, the excess of chlorinating agent and benzene was removed *in vacuo*. The residue was treated with ice-water and *pH* was adjusted to 6–7 with 16% aq. K_2CO_3. The precipitated solid was filtered off, washed with water and dried in the air. The crude product was extracted with hot methanol. The extract was decoloured with charcoal and concentrated. The crystalline precipitate was collected, recrystallized from methanol to produce white needles of pure **7**. Yield 1.3 g (30%), m.p. 139–140 °C.

^{1}H-NMR: $\delta = 7.40$ (d, 1 H, $J = 9$ Hz, 9-H), 7.42 (dd, 1 H, $J = 8$ Hz and 5 Hz, 3-H), 7.77 (s, 2 H, 5-H and 6-H), 8.03 (dd, 1 H, $J = 8$ Hz and 2 Hz, 4-H), 8.87 (dd, 1 H, $J = 5$ Hz and 2 Hz, 2-H), 9.27 (d, 1 H, $J = 9$ Hz, 10-H).

$C_{12}H_7N_2Cl$ (214.6).	Calcd.	C 67.1	H 3.3	N 13.0	Cl 16.5.
	Found	C 67.3	H 3.5	N 13.3	Cl 16.9.

2,8-Dichloro-1,7-phenanthroline (**8**)

1,7-Phenanthroline (**6**) di-N-oxide (4.0 g, 19 mmol) (obtained as reported in Ref. [7]) was added to a cooled solution of dry benzene (40 ml) and phosphorus oxychloride (18 ml). The mixture was gently refluxed for 45 min. Removal of the solvent and work up as described above afforded **8** in 28% yield. White needles, m.p. 169–172 °C (from methanol).

^{1}H-NMR: $\delta = 7.28$–7.46 (m, 2 H, 3-H and 9-H), 7.73 (s, 2 H, 5-H and 6-H), 7.95 (d, 1 H, $J = 9$ Hz, 4-H), 9.05 (d, 1 H, $J = 9$ Hz, 10-H).

$C_{12}H_6N_2Cl_2$ (248.9).	Calcd.	C 57.8	H 2.4	N 11.2	Cl 28.5.
	Found	C 57.6	H 2.5	N 11.3	Cl 27.8.

2,7-Dichloro-1,8-phenanthroline (**15**)

Compound **15** was obtained in 46% yield from di-N-oxide **14** (synthesized as reported in [8]) analogously as described above for **8**. M.p. 265–267 °C (from dimethylformamide).

^{1}H-NMR (*DMSO, TMS*): $\delta = 8.12$ (d, 1 H, $J = 7$ Hz, 3-H), 8.41 (d, 1 H, $J = 9$ Hz, 5-H), 8.53 (d, 1 H, $J = 9$ Hz, 6-H), 8.84 (d, 1 H, $J = 7$ Hz, 4-H), 8.85 (d, 1 H, $J = 6$ Hz, 9-H), 9.08 (d, 1 H, $J = 6$ Hz, 10-H).

$C_{12}H_6N_2Cl_2$ (248.9).	Calcd.	C 57.8	H 2.4	N 11.2	Cl 28.5.
	Found	C 57.9	H 2.35	N 11.3	Cl 28.7.

8-[2-(N,N-diethylamino)ethoxy]-1,7-phenanthroline (**9**)

To an oil-free sodium hydride (prepared from 1.45 g of 50% NaH, 30 mmol), anhydrous dimethyl sulfoxide (20 ml) and freshly distilled 2-(N,N-diethylamino)ethanol (4.0 ml, 30 mmol) were added and the mixture was stirred at 45 °C until all sodium hydride dissolved. After cooling to 20 °C, **7** (2.15 g, 10 mmol) was added and the mixture was stirred at room temperature for 20 h. Thereafter, the reaction mixture was poured into cold distilled water (100 ml). The crude ether separated as an oil, which was extracted with ethyl ether (4 × 50 ml), washed successively with water, dried over anhydrous magnesium sulfate and filtered. The filtrate was evaporated *in vacuo* to dryness.

The oil formed was dissolved in benzen (15 ml), filtered and after evaporation of the solvent it provided a pale yellow oil of pure base **9** (2.66 g, 90%).

^{1}H-NMR: $\delta = 1.06$ (t, 6 H, $J = 7$ Hz, —CH_3), 2.63 [q, 4 H, $J = 7$ Hz, —$N(CH_2)_2$—], 2.9 (t, 2 H, $J = 7$ Hz, —CH_2—N=), 4.56 (t, 2 H, $J = 7$ Hz, —OCH_2—), 7.03 (d, 1 H, $J = 9$ Hz, 9-H), 7.36 (dd, 1 H, $J = 8$ Hz and 5 Hz, 3-H), 7.8 (s, 2 H, 5-H and 6-H), 8.08 (dd, 1 H, $J = 8$ Hz and 2 Hz, 4-H), 8.88 (dd, 1 H, $J = 5$ Hz and 2 Hz, 2-H), 9.26 (d, 1 H, $J = 9$ Hz, 10-H).

Dihydrochloride of **9**

A solution of **9** (1.50 g, 5 mmol) in anhydrous ethyl ether (25 ml) was saturated with dry gaseous hydrogen chloride. The resulting white-yellow powder was filtered off, washed with cold benzene and dried at 95–100 °C for 1 h. Yield 1.41 g (72%), m.p. 190–193 °C.

IR (KBr): 2 440 and 2 640 (NH^+) cm^{-1}. UV (H_2O): $\lambda_{max} = 232$ nm ($\lg \varepsilon = 4.66$) and 276 (4.40).

$C_{18}H_{23}N_3OCl_2 \cdot H_2O$ (386.2).	Calcd.	C 56.0	H 6.0	N 10.9	Cl 18.3.
	Found	C 55.7	H 5.9	N 11.0	Cl 18.4.

2,8-Bis[2-(N,N-diethylamino)ethoxy]-1,7-phenanthroline (**10**)

This reaction was performed as described for ether **9**, with **8** (2.0 g, 8 mmol) and 2-(N,N-diethylamino)ethanol (6.34 ml, 48 mmol). Diether **10** was obtained in 56% yield as pale yellow oil.

^{1}H-NMR: $\delta = 1.0$ (t, 12 H, $J = 7$ Hz, —CH_3), 2.33–2.96 [m, 12 H, —$CH_2N(CH_2)_2$—], 4.0–4.63 (m, 4 H, —OCH_2—), 6.75 (d, 1 H, $J = 9$ Hz, 9-H), 6.9 (d, 1 H, $J = 9$ Hz, 3-H), 7.58 (s, 2 H, 5-H and 6-H), 7.78 (d, 1 H, $J = 9$ Hz, 4-H), 9.05 (d, 1 H, $J = 9$ Hz, 10-H).

Trihydrochloride of **10**

This reaction was performed as described for the previous salt with **10** (1.25 g, 3 mmol). Yield 1.52 g (93%), white powder, m.p. 350 °C (decomp.).

IR (KBr): 2480 and 2630 (NH^+) cm^{-1}. UV (H_2O): $\lambda_{max} = 234$ nm ($\lg \varepsilon = 4.56$) and 281 (4.38).

$C_{24}H_{37}N_4O_2Cl_3 \cdot H_2O$ (537.8).	Calcd.	C 53.6	H 7.3	N 10.4	Cl 19.7.
	Found	C 53.1	H 7.0	N 11.0	Cl 19.3.

2-[2-(N,N-diethylamino)ethoxy]-1,10-phenanthroline (**3**)

This reaction was performed as above for **9** with **1** (1.40 g, 6.5 mmol) and 2-(N,N-diethylamino)ethanol (2.58 ml, 19.5 mmol). Ether **3** was obtained in 20% yield as colourless oil.

^{1}H-NMR: $\delta = 1.03$ (t, 6 H, $J = 7$ Hz, CH_3), 2.60 [q, 4 H, $J = 7$ Hz, —$N(CH_2)_2$—], 2.9 (t, 2 H, $J = 7$ Hz, —CH_2N=), 4.76 (t, 2 H, $J = 7$ Hz, —OCH_2—), 7.01 (d, 1 H, $J = 9$ Hz, 3-H), 7.33 (dd, 1 H, $J = 8$ Hz and 5 Hz, 8-H), 7.53 (s, 2 H, 5-H and 6-H), 7.86 (d, 1 H, $J = 9$ Hz, 4-H), 8.0 (dd, 1 H, $J = 8$ Hz and 2 Hz, 7-H), 9.0 (dd, 1 H, $J = 5$ Hz and 2 Hz, 9-H).

2,9-Bis[2-(N,N-diethylamino)ethoxy]-1,10-phenanthroline (**4**)

This reaction was performed as described for **10** with **2** (2.5 g, 10 mmol) and 2-(N,N-diethylamino)ethanol (7.93 ml, 60 mmol). Diether **4** was obtained in 97% yield as colourless oil.

^{1}H-NMR: $\delta = 1.07$ (t, 12 H, $J = 7$ Hz, —CH_3), 2.65 [q, 8 H, $J = 7$ Hz, —$N(CH_2)_2$—], 2.98 (t, 4 H, $J = 7$ Hz, —CH_2N=), 4.75 (t, 4 H, $J = 7$ Hz, —OCH_2—), 6.96 (d, 2 H, $J = 9$ Hz, 3-H and 8-H), 7.50 (s, 2 H, 5-H and 6-H), 7.96 (d, 2 H, $J = 9$ Hz, 4-H and 7-H).

Dihydrochloride of **3**

This reaction was performed as described for the previous salt from **3** (1.2 g, 4 mmol). Yield 1.66 g (94%). White powder, m.p. 202–204 °C.

IR (KBr): 2485 and 2670 (NH^+) cm^{-1}. UV (H_2O): $\lambda_{max} = 222$ nm ($\lg \varepsilon = 4.70$) and 274 (4.56).

$C_{18}H_{23}N_3OCl_2$ (368.2).	Calcd.	C 58.7	H 6.2	N 11.4	Cl 19.2.
	Found	C 58.5	H 6.3	N 11.8	Cl 19.7.

Trihydrochloride of **4**

This salt was obtained in the same manner as the dihydrochloride of **9**. Yield 75%. White needles, m.p. 195–197 °C (from mixture ethyl acetate-ethanol, 2 : 1). UV (H_2O): $\lambda_{max} = 227$ ($\lg \varepsilon = 4.59$) and 279 (4.33).

$C_{24}H_{37}N_4O_2Cl_3 \cdot H_2O$ (537.8). Calcd. C 53.6 H 7.3 N 10.4 Cl 19.7.
Found C 53.8 H 7.1 N 10.5 Cl 19.7.

2,7-Bis[2-(N,N-diethylamino)ethoxy]-1,8-phenanthroline (**13**)

This compound was obtained from **15** (2.0 g, 8 mmol) and 2-(N,N-diethylamino)ethanol (6.34 mml, 48 mmol) in the same manner as **10**. Diether **13** was obtained in 52% yield as colourless oil.

^{1}H-NMR: $\delta = 1.10$ (t, 12 H, $J = 7$ Hz, —CH_3), 2.36–2.96 [m, 12 H, —$CH_2N(CH_2)_2$—], 4.53 (t, 4 H, $J = 7$ Hz, —OCH_2—), 6.88 (d, 1 H, $J = 9$ Hz, 3-H), 7.43 (d, 1 H, $J = 9$ Hz, 5-H), 7.78 (d, 1 H, $J = 9$ Hz, 4-H), 7.88 (d, 1 H, $J = 9$ Hz, 6-H), 8.08 (d, 1 H, $J = 6$ Hz, 9-H), 8.24 (d, 1 H, $J = 6$ Hz, 10-H).

Trihydrochloride of **13**

This salt was obtained in the same manner as the dihydrochloride of **9** in 90% yield. White powder, m.p. 167–169 °C.

IR (KBr): 2485 and 2650 (NH^+) cm^{-1}. UV (H_2O); $\lambda_{max} = 228$ nm ($\lg \varepsilon = 4.38$), 252 (4.30), 268 (4.25) and 297 (3.73).

$C_{24}H_{37}N_4O_2Cl_3 \cdot H_2O$ (537.8). Calcd. C 53.6 H 7.3 N 10.4 Cl 19.7.
Found C 53.3 H 7.0 N 10.6 Cl 19.4.

7-[2-(N,N-diethylamino)ethoxy]-1,8-phenanthroline (**12**)

Monoether **12** was obtained from **11** (obtained as described in Ref. [8]) (1.40 g, 6.5 mmol) and 2-(N,N-diethylamino)ethanol (2.58 ml, 19.5 mmol) in the same manner as **9**. Column chromatography on silica gel, with methanol containing 20% of ethyl acetate as eluent, gave monoether **12** (light brown oil, 0.60 g, 31%) in the first fractions, and diether **13** (colourless oil, 0.42 g, 15%) in the following ones.

^{1}H-NMR (*TMS*): $\delta = 1.08$ (t, 6 H, $J = 7$ Hz, —CH_3), 2.40–3.0 [m, 6 H, —$CH_2N(CH_2)_2$—], 4.57 (t, 2 H, $J = 7$ Hz, —OCH_2—), 7.39 (dd, 1 H, $J = 5$ Hz and 8 Hz, 3-H), 7.48 (d, 1 H, $J = 9$ Hz, 5-H), 7.56 (d, 1 H, $J = 9$ Hz, 6-H), 8.1 (dd, 1 H, $J = 8$ Hz and 2 Hz, 4-H), 8.22 (d, 1 H, $J = 6$ Hz, 9-H), 8.58 (d, 1 H, $J = 6$ Hz, 10-H), 8.92 (dd, 1 H, $J = 5$ Hz and 2 Hz, 2-H).

Dihydrochloride of **12**

This salt was obtained in the same manner as the dihydrochloride of **9** in 87% yield. White powder, m.p. 176–179 °C.

IR (KBr): 2490 and 2595 (NH^+) cm^{-1}. UV (H_2O): $\lambda_{max} = 227$ nm ($\lg \varepsilon = 4.54$), 248 (4.33), 254 (4.35), 265 (4.30) and 297 (3.74).

$C_{18}H_{23}N_3OCl_2 \cdot H_2O$ (386.2). Calcd. C 56.0 H 6.0 N 10.9 Cl 18.3.
Found C 55.8 H 6.11 N 11.4 Cl 18.1.

3-[2-(N,N-diethylamino)ethoxy]-4,7-phenanthroline (**18**)

Monoether **18** was obtained from **16** and 2-(N,N-diethylamino)ethanol in the same manner as **9**. The crude base **18** was recrystallized from a mixture of *n*-hexane : ethyl acetate (2 : 1) to give pure **18** in 45% yield as white microcrystalline solid, m.p. > 206 (decomp) °C.

^{1}H-NMR: $\delta = 1.0$ (t, 6H, $J = 7$ Hz, —CH_3), 2.50 (q, 4H, $J = 7$ Hz, —CH_2—), 2.73 (t, 2H, $J = 7$ Hz, —CH_2N=), 4.40 (t, 2H, $J = 7$ Hz, —OCH_2—), 6.78 (d, 1H, $J = 8$ Hz, 2-H), 7.21 (dd, 1H, $J = 8$ Hz and 5 Hz, 9-H), 7.88 (d, 2H, $J_{AB} = 5$ Hz, 5-H and 6-H), 8.3 (d, 1H, $J = 8$ Hz, 1-H), 8.35 (dd, 1H, $J = 8$ Hz and 2 Hz, 10-H), 8.71 (dd, 1H, $J = 5$ Hz and 2 Hz, 8-H).

Dihydrochloride of **18**

This salt was obtained in the same manner as the dihydrochloride of **9**. Recrystallization from a mixture of ethanol : ethyl acetate (1 : 2) gave pure dihydrochloride of **18** in 82% yield as white microcrystalline solid. This salt heated on a *Böetius* apparatus in the range of temperature 165–178 °C reset into small needles, which mèlted at 216 °C and again, above 223 °C, solidified affording needles, melting slowly with decomposition above 258 °C.

IR (KBr): 2485 and 2590 (NH^+) cm^{-1}. UV (H_2O): $\lambda_{max} = 234$ nm (lg $\varepsilon = 4.66$) and 280 (4.34).

$C_{18}H_{23}N_3OCl_2 \cdot H_2O$ (386.2). Calcd. C 56.07 H 6.00 N 10.9 Cl 18.3.
Found C 55.8 H 5.84 N 10.75 Cl 18.2.

3,8-Bis[2-(N,N-diethylamino)ethoxy]-4,7-phenanthroline (**19**)

Free base of vivakorfen was obtained from **17** and 2-(N,N-diethylamino)ethanol in the same manner as **10**. Crude **10** was filtered off, washed with cold water, and dried in the air. Recrystallization from frozen *n*-hexane gave pure **19**, in 72% yield, as white plates, m.p. 66–67, 5 °C.

^{1}H-NMR: $\delta = 1.0$ (t, 12H, $J = 7$ Hz, —CH_3), 2.50 (q, 8H, $J = 7$ Hz, —CH_2—), 2.73 (t, 4H, $J = 7$ Hz, —CH_2N=), 4.38 (t, 4H, $J = 7$ Hz, —OCH_2), 6.75 (d, 2H, $J = 8$ Hz, 2-H and 9-H), 7.76 (s, 2H, 5-H and 6-H), 8.35 (d, 2H, $J = 8$ Hz, 1-H and 10-H).

Dihydrochloride of **19**

A solution of base **19** (1.65 g, 4 mmol) in a mixture of anhydrous ethyl ether : ethanol (15 : 1) was saturated with dry gaseous hydrogen chloride. The resulting white powder was filtered off, washed with cold ethyl acetate and dried at 85 °C for 1 h. Recrystallization from a mixture of anhydrous ethanol : ethyl acetate (1 : 3) gave analytically pure dihydrochloride of **19** (1.5 g, 78%) as white needles, m.p. 197–199 °C.

IR (KBr): 2490 and 2600 (NH^+) cm^{-1}. ^{1}H-NMR (D_2O): $\delta = 1.6$ (t, 12H, $J = 7$ Hz, —CH_3), 3.63 (q, 8H, $J = 7$ Hz, —CH_2—), 3.83–4.08 (m, 4H, —CH_2N=), 4.83 (m, D_2O + —OCH_2—), 7.3 (d, 2H, $J = 8$ Hz, 2-H and 9-H), 7.63 (s, 2H, 5-H and 6-H), 8.6 (d, 2H, $J = 8$ Hz, 1-H and 10-H).

$C_{24}H_{36}N_4O_2Cl_2$ (483.4). Calcd. C 59.5 H 7.4 N 11.5 Cl 14.6.
Found C 59.6 H 7.5 N 11.4 Cl 14.9.

3,8-Bis[3-(N,N-diethylamino)propoxy]-4,7-phenanthroline (**20**)

This compound was obtained from **17** and 3-(N,N-diethylamino)propanol in the same manner as **10**. The crude base **20** was recrystallized from frozen *n*-hexane to give pure diether in 60% yield as white microcrystalline plates, m.p. 75–76 °C.

^{1}H-NMR: $\delta = 0.93$ (t, 12 H, $J = 7$ Hz, —CH_3), 1.83 (qu, 4 H, $J = 7$ Hz, —CH_2—), 2.16–2.66 [m, 12 H, —$N(CH_2)_3$], 4.35 (t, 4 H, $J = 7$ Hz, —OCH_2—), 6.75 (d, 2 H, $J = 8$ Hz, 2-H and 9-H), 7.76 (s, 2 H, 5-H and 6-H), 8.3 (d, 2 H, $J = 8$ Hz, 1-H and 10-H).

Dihydrochloride of **20**

This salt was obtained in the same manner as the dihydrochloride of **9** in 87% yield. White microcrystalline solid, m.p. > 206 °C (decomp.) [from a mixture of ethyl acetate : ethanol (3 : 1)].

IR (KBr): 2485 and 2595 (NH^+) cm^{-1}. UV (H_2O): $\lambda_{max} = 239$ nm ($\lg \varepsilon = 4.78$) and 293 (4.38).

$C_{26}H_{40}N_4O_2Cl_2$ (511.5). Calcd. C 61.0 H 7.8 N 10.9 Cl 13.8.
Found C 59.7 H 8.0 N 11.0 Cl 13.5.

3,8-Bis[5-(N,N-diethylamino)pentoxy]-4,7-phenanthroline (**21**)

This compound was obtained from **17** and 5-(N,N-diethylamino)pentanol in the same manner as **10**. Crude base **21** was recrystallized from frozen *n*-hexane to give pure diether **21**, in 58% yield, as white microcrystalline plates, m.p. 59–61 °C.

^{1}H-NMR: $\delta = 1.0$ (t, 12 H, $J = 7$ Hz, —CH_3), 1.42–1.94 [m, 12 H, —$(CH_2)_3$—], 2.44 (q, 8 H, $J = 7$ Hz, —CH_2CH_3), 2.58 (t, 4 H, $J = 7$ Hz, —CH_2N=), 4.48 (t, 4 H, $J = 7$ Hz, —OCH_2—), 6.96 (d, 2 H, $J = 8$ Hz, 2-H and 9-H), 7.96 (s, 2 H, 5-H and 6-H), 8.52 (d, 2 H, $J = 8$ Hz, 1-H and 10-H).

Dihydrochloride of **21**

This salt was obtained in the same manner as the dihydrochloride of **9** in 79% yield. White microcrystalline solid, m.p. 128–130 °C [from a mixture of ethyl acetate : ethanol (3 : 1)].

UV (H_2O): $\lambda_{max} = 239$ nm ($\lg \varepsilon = 4.83$) and 294 (4.43).

$C_{30}H_{48}N_4O_2Cl_2$ (567.6). Calcd. C 63.5 H 7.1 N 9.8 Cl 12.5.
Found C 63.7 H 6.9 N 10.0 Cl 12.9.

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