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To Be Dinuclear or Not: Z-DNA Induction by Nickel Complexes

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Abstract: The left-handed Z-DNA has been identified as a gene regulating element. Therefore the generation of Z-DNA through metal complexes might be an innovative way for the regulation of gene expression. Use of the new dinuclear complex *N*,*N*,*N*′,*N*′-tetrakis-[2-(3,5-dimethylpyrazol-1-yl)ethyl]-1,3-propylenediamine-bis(nickel(II) dinitrate) (**2**) reversibly induced Z-DNA formation. However, when a 1:1 ratio of metal/dinucleating ligand was used as a control, the midpoint of the B- to Z-DNA transition was at the same nickel concentration as in case of the dinuclear complex. The novel mononuclear analogue, *N*-methyl-*N*,*N*-bis-[2-(3,5-dimethylpyrazol-1-yl)ethyl]amine-nickel(II)-dinitrate (**3**) was inducing the Z-DNA at a similar ratio versus nucleotides as free nickel(II) itself. For the first time, proton and nickel binding constants for the bis-[2-(pyrazol-1-yl)ethyl]amine ligand system are reported and discussed. Both nickel complexes **2** and **3** were structurally characterized by single crystal analysis. Furthermore, the synthesis of the two new ligands, *N*,*N*,*N*′,*N*′-tetrakis-[2-(3,5-dimethylpyrazol-1-yl)ethyl]-1,2-propylenediamine (**4**) and *N*-methyl-*N*,*N*-bis-[2-(3,5-dimethylpyrazol-1-yl)ethyl]amine (**5**) is described. The two major synthetic pathways leading to polypyrazoyl amines in general are critically discussed with respect to yield, reproducibility and handling of the intermediates.

Keywords: bioinorganic chemistry · DNA structures · metal complexes · N ligands · nickel

Introduction

The binding of first- or second-row transition metal complexes to nucleobases has been studied for a long time. In most cases, a hydrophobic ligand binds to one of the DNA grooves or an extended aromatic system of the metal complex intercalates between the base pairs.[1,2] Contrarily, the inner-sphere coordination of first- or second-row transition metals to either phosphates and/or nucleobases has been studied less intensively.[3–5] Here, the subsequent reactions after the initial binding, such as phosphate diester hydrolysis[6] or oxidation promoted by metal complexes,[7] are of special interest.

The left-handed Z-form of oligonucleotides was first observed at high salt concentrations, but in the mean time, transition metals[8] as well as proteins[9] were also found to induce the Z-form. Since Z-DNA was found to be a gene regulating element,[10] our interest focused on the conditions which lead to its formation starting from B-DNA. In Z-DNA, the N7 of guanine is exposed to the solvent.[11] Binding of nickel ions[12] or nickel complexes[13] to this atom induces the conformational change from B- to Z-DNA. During our studies aimed at a better understanding the factors which influence the formation of the left-handed Z-DNA,[12] we proposed that both metal centres of a dinuclear complex might act in a synergistic way to induce Z-DNA at a lower metal-to-nucleobase ratio than their mononuclear analogues.[14,15] This concept has been realized in our recent work of bis-12[ane]N3 complexes.[16] The ideal dinuclear system to induce Z-DNA should have the following properties:

- each metal cation should have at least one labile coordination site, thereby allowing inner-sphere binding;
- the metal-to-metal distance should be between 5 and 7 Å;
- the bridging ligand with **2** should contain sterically rather demanding chelating moieties to enforce preferential selectivity for Z-DNA versus B-DNA.

Following a search on the Cambridge Structure Database,[17] we selected dinickel complex **1** as an additional promising

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compound fulfilling the above-mentioned criteria. The Ni–Ni distance in the crystal structure of **1** was 6.453(1) Å,[18] though this value was expected to change upon rotation of the ligand to accommodate the *syn* coordination of the metals to the DNA major groove. In order to further explore the chemistry of complexes such as **1**, we synthesised the new dinuclear complex **2** and for comparison the mononuclear complex **3** (Figure 1). They are based on the novel

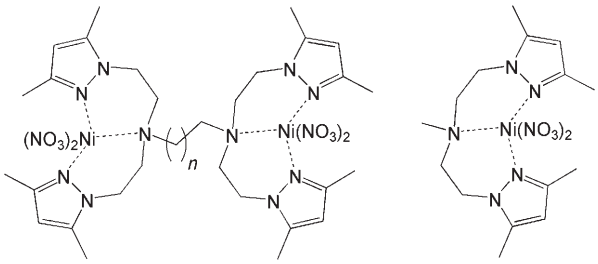
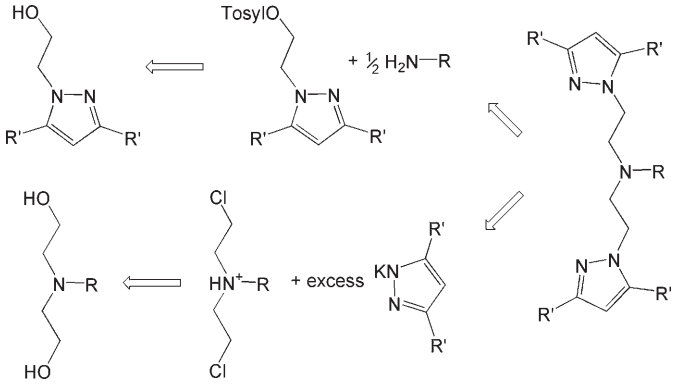


Figure 1. Nickel complexes studied.

dinucleating ligand *N*,*N*,*N*′,*N*′-tetrakis-[2-(3,5-dimethylpyrazol-1-yl)ethyl]1,2-propylenediamine (**4**) and the new mononucleating ligand *N*-methyl-*N*,*N*-bis-[2-(3,5-dimethylpyrazol-1-yl)ethyl]amine (**5**). In this paper, we report the synthesis and characterisation of compounds **2**–**5**, as well as their reversible interactions with poly d(GC) with respect to the B- to Z-DNA transition. For the first time, proton and nickel binding constants of the bis-[2-(pyrazol-1-yl)ethyl]amine ligand system are reported and discussed. In addition, we critically evaluate the various synthetic pathways leading to polypyrazoyl ligands such as **4** and **5**.

Results and Discussion

Two different synthetic pathways leading to bis-[2-(pyrazol-1-yl)ethyl]amines have been described in the literature thus far (Scheme 1).[19–21] 2-(3,5-Dimethylpyrazol-1-yl)ethanol was transformed into its tosylate derivative, which was then used to alkylate aliphatic amines.[19] We first followed this route, but found that the yield of the tosylation step was rather low and the amine alkylation was not reproducible. Thus we turned to another approach starting from diethanolamine derivatives. These were treated with thionyl chloride to yield aza analogues of mustard gas. As described in the literature,[20,21] the bis-(2-chloroethyl) ammonium salts were not isolated as their free bases, but



Scheme 1. Retrosynthetic analysis for bis-[2-(pyrazol-1-yl)ethyl]amines.

instead directly reacted with an excess of potassium pyrazolate. This procedure had two advantages: the highly vesicant bis-(2-chloroethyl) ammonium salts are much less volatile than their free bases and the formation of side products such as piperazine derivatives through dimerisation was completely suppressed.

The synthetic route for bis-(2-chloroethyl)amine turned out to be highly efficient and reproducible. The new ligands *N*,*N*,*N*′,*N*′-tetrakis-[2-(3,5-dimethylpyrazol-1-yl)ethyl]1,2-propylenediamine (**4**) and *N*-methyl-*N*,*N*-bis-[2-(3,5-dimethylpyrazol-1-yl)ethyl]amine (**5**) were synthesized on a multigramm scale from commercial available chemicals in two and three steps in an overall yield of 86 and 72%, respectively. The ligands were treated with two or one equivalents of $Ni(NO_3)_2$ to yield compounds **2** and **3**. In both cases, single crystals were grown by vapor diffusion of methanolic solutions against tetrahydropyran. Both structures show a distorted octahedral coordination geometry around the nickel centers (Figures 2 and 3). The distortion is mainly caused by the chelating mode of a nitrate molecule. As described before for dinickel complex **1**, the bis-[2-(3,5-dimethylpyrazol-1-yl)ethyl]amine units coordinate to the metal in a facial fashion.[18] The Ni–Ni distance of 6.7698(6) Å in **2** is a little bit longer than in the case of the ethylene bridged **1** (6.453(1) Å).

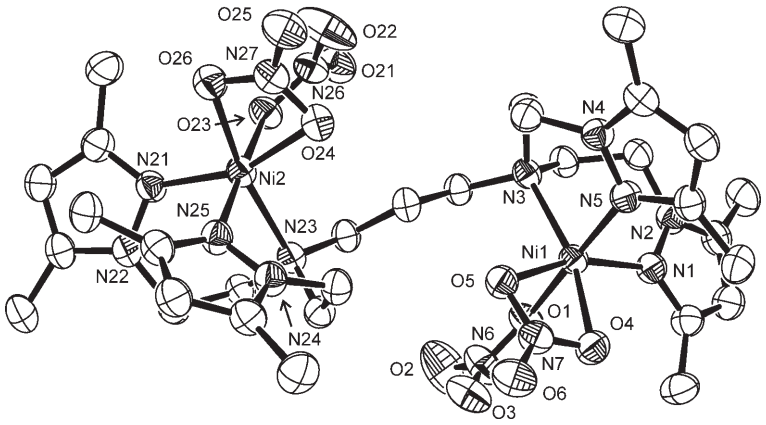


Figure 2. ORTEP plot of **2**·($C_5H_{10}O$)·(C_2H_3N), ellipsoids drawn at 50% probability. Hydrogen atoms and solvent molecules were omitted for clarity.

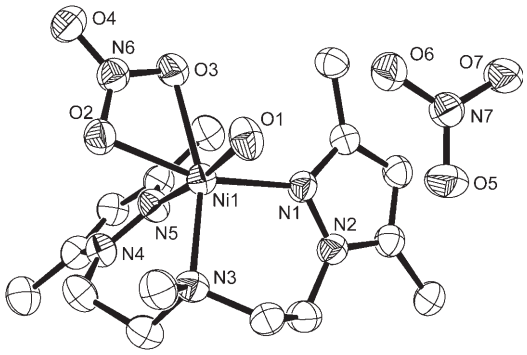


Figure 3. ORTEP plot of **3**·H_2O, ellipsoids drawn at 50% probability. Hydrogen atoms were omitted for clarity

Both complexes **2** and **3** as well as ligands **4** and **5** were tested for their ability to induce Z-DNA formation by titrating poly d(GC) with solutions containing either **2**, **3**, **4** or **5**. As a control as to whether the dinuclear complex **2** remains intact, the 1:1 adduct of $Ni(NO_3)_2$ and **4** was also tested. The transformation from B- to Z-DNA was followed by circular dichroism (CD) and UV/Vis spectroscopy at room temperature at a pH 7. Addition of complexes **2** and **3** as well as the 1:1 adduct of $Ni(NO_3)_2$ and **4** (Figure 4 and Figure S1 in the

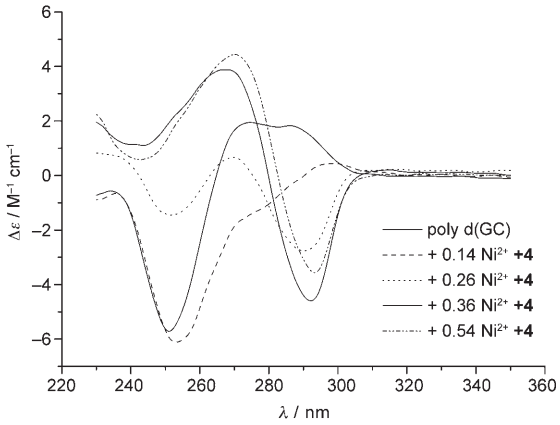


Figure 4. CD spectra showing the B- to Z-DNA transition induced by the 1:1 adduct of $Ni(NO_3)_2$ and **4**. Conditions: 1 mM sodium cacodylate, 0.1 mM poly d(GC).

Supporting Information), but not the ligands **4** and **5** (Figure S2, Supporting Information), induced Z-DNA with its characteristic positive band at 269 nm and a negative band at 293 nm.[22] The transition from B- to Z-DNA was followed at 255 nm (Figure 5). The midpoint of the transitions, expressed in units of metal ions per DNA phosphates,[23] were determined to be 0.26(1) for **2**, 0.28(1) for the 1:1 adduct of $Ni(NO_3)_2$ and **4**, and 0.49(1) for **3** (Table 1). Mononuclear complex **3** showed a comparable efficiency for Z-DNA induction as $Ni(NO_3)_2$ which had a midpoint of the transition at 0.48 equivalents.[12] The preformed dinuclear complex **2** and the 1:1 adduct of $Ni(NO_3)_2$ both induced the Z-DNA at half the nickel concentration compared with nickel nitrate alone. A model to explain this behaviour would include that

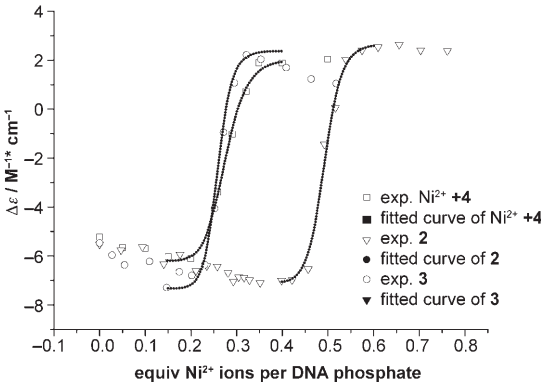


Figure 5. Experimental CD titration of poly d(GC) with either **2**, **3** or the 1:1 adduct of $Ni(NO_3)_2$ and **4** followed at 255 nm together with the calculated fitting curves for the B- to Z-DNA transition.

Table 1. Induction of Z-DNA by $Ni(NO_3)_2$, **2**, 1:1 adduct of $Ni(NO_3)_2$ and **4**, **3**, **4**, or **5**.

Compound	Equivalents of metals/ligands needed for the midpoint of the transition from B- to Z-DNA
$Ni(NO_3)_2$	0.48[12]
$[Ni_2(NO_3)_4(\mathbf{4})] \equiv \mathbf{2}$[a]	0.26(1)
$[Ni(NO_3)_2]/\mathbf{4}$[b] 1:1	0.28(1)
$[Ni(NO_3)_2(\mathbf{5})] \equiv \mathbf{3}$[a]	0.49(1)
4	no transition
5	no transition

[a] Preformed complexes. [b] Formed in situ, before addition to the DNA.

in water at a submillimolar concentration complex **2** is loosing one of its two nickel cations to yield a 1:1 nickel/ligand **4** complex. The better efficiency of either **2** or the 1:1 adduct of $Ni(NO_3)_2$ and **4** might come from the formation of macrochelate complex of nickel coordinating to N7 and the ternary free amine of the previous second binding side, which is protonated at pH 7 (Figure 6). An UV/Vis titration was attempted to directly study the behaviour of complex **2** in the same aqueous solution, in which the circular dichroism studies were performed (1 mM cacodylate buffer, pH 7). However, the poor solubility of ligand **4** in water and the low extinction coefficient of complex **2** impeded our efforts to independently study its identity at the submillimolar concentrations of the CD experiment. Pettinari and co-workers have studied the UV/Vis properties of bispyrazoylmethane metal complexes in the organic solvents, such as acetonitrile.[24]

Additional amounts of complex **2** or the 1:1 adduct of $Ni(NO_3)_2$ and **4** to poly d(GC) already in the Z-form transformed the DNA to yet another known left-handed conformation, previously denoted as either Z′- or U-DNA (Figures 4 and 5). After addition of total 0.7 equivalents of **2**, no further change in the CD signal could be detected. The transition from Z- to Z′-DNA, with its characteristic isosbestic

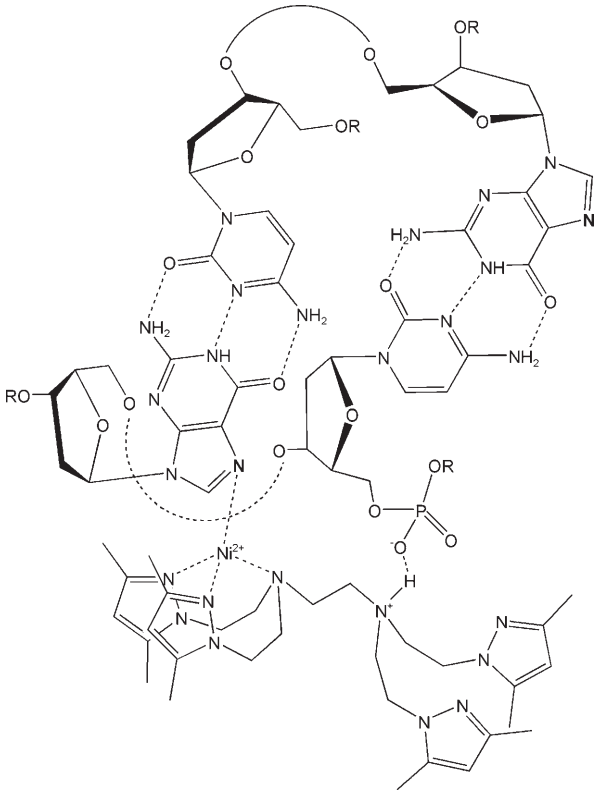


Figure 6. Postulated interaction between Z-DNA and the 1:1 adduct of Ni^{2+} and **4**.

point at 268 nm and bathochromic shift of 5 nm of the positive signal at around 270 nm, had previously been observed when concentrations of either cobalt hexammine or ethanol were increased.[25–27] The formation of Z-DNA could be reversed by addition of a slight excess of EDTA (Figure S3, Supporting Information).

To gain further insight in the behaviour of ligands **4** and **5**, we studied their proton and nickel binding constants. Due to the limited solubility of the ligands at submillimolar concentrations in pure aqueous electrolyte solutions, experiments were performed in 40% isopropanol for **4** and 40% methanol for **5** as organic cosolvents. The results of the titrations are shown in Table 2. Both ligands are coordinating the nickel rather weakly under the experimental conditions.

Table 2. Proton and nickel binding constants of **4** and **5**.

	4	**5**
proton binding constants	$\beta_1 = 10.56 \pm 0.02$ $\beta_2 = 16.85 \pm 0.03$ $\beta_3 = 20.87 \pm 0.04$ $\beta_4 = 24.0 \pm 0.1$	$\beta_1 = 6.10 \pm 0.02$ $\beta_2 \approx 8.1$
nickel binding constants	$\beta_1 = 4.5 \pm 0.1$ (product: $[Ni(\mathbf{4})]^{2+}$) $\beta_2 = 12 \pm 0.5$ (product: $[NiH(\mathbf{4})]^{3+}$)	$\beta_1 = 2.8 \pm 0.2$ (product: $[Ni(\mathbf{5})]^{2+}$)

Conclusion

Two new ligands *N*,*N*,*N*′,*N*′-tetrakis-[2-(3,5-dimethylpyrazol-1-yl)ethyl]-1,2-propylenediamime (**4**) and *N*-methyl-*N*,*N*-bis-[2-(3,5-dimethylpyrazol-1-yl)ethyl]amine (**5**) were synthesized on a multigram scale from commercially available chemicals in two and three steps in an overall yield of 86 and 72%, respectively. The synthetic pathway via the *N*,*N*-bis-(2-chloroethyl)ammonium salts proved to be not only high-yielding but also perfectly reproducible. These two advantages, not present in the alternative synthetic path via the tosylate route (Scheme 1), outweighed the handling of the reactive intermediates.

Two novel complexes have been described herein: dinuclear *N*,*N*,*N*′,*N*′-tetrakis-[2-(3,5-dimethylpyrazol-1-yl)ethyl]-1,3-propylenediamine-bis(nickel(II) dinitrate) (**2**) and its mononuclear analogue, *N*-methyl-*N*,*N*-bis-[2-(3,5-dimethylpyrazol-1-yl)ethyl]amine-nickel(II) dinitrate (**3**). Z-DNA could be induced by either using the preformed dinuclear complex **2** or the 1:1 adduct of $Ni(NO_3)_2$ at the half the nickel concentration of either nickel nitrate or the mononuclear complex **3** alone. Determination of the proton and nickel binding constants of **4** and **5** indicated that they are rather weakly coordinating to nickel. From these results, it is difficult to predict an active species in case of **4** for the ternary system DNA–nickel–ligand **4**. One possibility is that complex **2** looses one of its two nickel cations to yield a 1:1 nickel/ligand **4** complex. The better efficiency of either **2** or the 1:1 adduct of $Ni(NO_3)_2$ and **4** versus either Ni^{2+} alone or complex **3** was explained by the formation of a macrochelate of the nickel coordinating to N7 of guanosine and a hydrogen bridge between a DNA phosphate and the ternary protonated amine of the former second binding side of ligand **4** (Figure 6). Higher concentrations of either **2** or the 1:1 adduct of $Ni(NO_3)_2$ and **4** transformed poly d(GC) to the rare Z′-DNA conformation.

Experimental Section

General: All chemicals except poly d(GC) were purchased from Aldrich or Fluka (Buchs, CH) and used without further purification. Poly d(GC) was purchased from GE Healthcare (formerly Amersham Biosciences) and used as received. The ligand syntheses were performed under a nitrogen atmosphere. The reactions were monitored by thin-layer chromatography (TLC) on 0.25 mm Merck silica gel aluminium plates (60 F_{254}) or aluminium oxide pre-coated plastic sheets (alox N/UV_{254}) using UV light or Schlittler reagent[28] as a visualising agent. Column chromatography was performed on silica gel (particle size 0.040–0.063 mm) or aluminium oxide (basic, 0.05–0.15 mm, pH 9.5 ± 0.5). Elemental analyses were performed on a Leco CHNS-932 elemental analyser. Electrospray Ionisation (ESI) mass spectra were recorded on a Merck Hitachi M-8000 spectrometer. NMR spectra were recorded on a Varian Mercury 200 MHz or Gemini 300 MHz spectrometer. The chemical shifts are relative to residual solvent protons as reference.

Circular dichroism measurements were performed using a Jasco J-810 spectropolarimeter equipped with a Jasco PFD-4255 Peltier temperature controller. The spectra were smoothed with the help of the program ORIGIN. The calculation of the transition midpoints was done following

the Hill equation. y_0 and y_∞ were set to the minimum and maximum spectrocopic responses of the observed transition.[29,30]

Crystallography: Crystallographic data were collected on a Stoe IPDS diffractometer at 183(2) K using a graphite-monochromated $Mo_{K\alpha}$ radiation ($\lambda = 0.71073$ Å). Suitable crystals were covered with Paratone N oil, mounted on top of a glass fiber and immediately transferred to the diffractometer. Eight thousand reflections distributed over the whole limiting sphere were selected by the program SELECT and used for unit cell parameter refinement with the program CELL.[31] Data were collected for Lorentz and polarisation effects as well as for absorption (numerical). Structures were solved with direct methods using SHELXS-97[32] or SIR97[33] and were refined by full-matrix least-squares methods on F^2 with SHELXL-97.[34] The program PLATON was used to check whether higher symmetry was present.[35] Selected crystallographic data is shown in Table 3.

Table 3. Crystallographic data of **2**·$C_5H_{10}O$·C_2H_3N and **3**·H_2O.

	2·$C_5H_{10}O$·C_2H_3N	**3**·H_2O
formula	$C_{38}H_{63}N_{15}Ni_2O_{13}$	$C_{15}H_{26}N_7NiO_7$
M_r	1055.45	475.14
color/habit	blue plate	blue plate
crystal system	triclinic	monoclinic
space group	$P\bar{1}$	$P2_1$
a [Å]	8.5748(6)	8.4648(4)
b [Å]	15.570(1)	9.5669(6)
c [Å]	19.0251(12)	13.0389(6)
α [°]	77.975(7)	90
β [°]	83.622(8)	95.863(5)
γ [°]	88.939(8)	90
V [Å^3]	2468.3(3)	1050.4(1)
Z	2	2
ρ_{calcd} [g cm^{-3}]	1.420	1.502
μ [mm^{-1}]	0.837	0.976
reflections measured	13616	3205
reflections observed [$I > 2\sigma(I)$]	10595	2631
Flack parameter	–	0.07(2)
R_1/wR_2 [$I > 2\sigma(I)$]	0.0555/0.1639	0.0461/0.1371

CCDC-282007 and -282008 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Synthesis of mono- and dinucleating ligands

***N,N,N′,N′*-Tetrakis-(2-hydroxyethyl)-1,3-diamino-propane (6)**:[20] 1,3-Dibromopropane (80 mmol, 16.15 g) and *N*,*N*-diethanolamine (160 mmol, 16.61 g) were added to a round bottom flask containing a a solution of K_2CO_3 (80 mmol, 11.06 g) in ethanol (150 mL). The mixture was stirred and heated under reflux for 64 h. After cooling down to room temperature, chloroform (50 mL) was added and the solution was stirred for another 12 h. The mixture was filtered and the solid precipitate was thoroughly washed with chloroform. The combined filtrates were concentrated in vacuo to give a colorless oil. The crude material was purified by chromatography on silica gel (methanol with 10% concentrated ammonia/dichloromethane 1:4). Fractions with an R_f value of 0.34 were combined to yield the product (16.95 g, 67.7 mmol, 85%). ^{1}H NMR (200 MHz, [D$_4$]MeOH, 25 °C): $\delta = 1.67$ (br quint, 2H, $CH_2CH_2CH_2$), 2.67 (m, 12H, NCH_2), 3.62 ppm (t, $^3J = 5.8$ Hz, 8H, CH_2OH); ^{13}C NMR (50 MHz, [D$_4$]MeOH, 25 °C): $\delta = 25.21$ ($CH_2CH_2CH_2$), 54.06 ($CH_2CH_2CH_2$), 57.63 (CH_2CH_2OH), 60.73 ppm (CH_2OH); ESI-MS: *m*/*z* (%): 250 (100) [M^+]; elemental analysis calcd (%) for $C_{11}H_{26}N_2O_4$·H_2O: C 49.23, H 10.52, N 10.44; found: C 49.38, H 10.87, N 10.15.

***N,N,N′,N′*-Tetrakis-(2-chloroethyl)-1,3-propylene-diammonium dichloride (7)**: Compound **6** (12 mmol, 3 g) was dissolved in toluene (5 mL) and added dropwise during 1 h to a solution of thionyl chloride (54 mmol, 6.42 g) in toluene (30 mL). The mixture was stirred and heated under reflux for 2 h. The solvent was removed in vacuo. No analysis was made due to the high reactivity of this nitrogen mustard analogue. The crude material was used without further purification.

WARNING: Analogues of *N*,*N*-di-(2-chloroethyl)amines are highly toxic and powerful vesicants! Cleaning of all glassware exposed to every reaction product was done with a 3% solution of $Na_2S_2O_3$ in water.[20,36]

***N,N,N′,N′*-Tetrakis-[2-(3,5-dimethylpyrazol-1-yl)ethyl]-1,2-propylenediamime (4)**: A solution of 3,5-dimethylpyrazole (6.06 g, 63 mmol) in dry diethyleneglycol dimethylether (100 mL) was stirred with potassium (2.5 g, 63 mmol) at 70 °C until all metal was dissolved.[37] Compound **7** (4.05 g, 12.6 mmol) was added to this solution at 25 °C and the resulting solution was stirred at 110 °C for 4 d. The solvent was removed on a rotary evaporator, final traces of solvent were removed by adding water to the oil and reducing the volume in vacuo. The compound was purified by dissolving the crude material in dichloromethane, filtering through Celite and washing with ethanol. Excess pyrazole was removed by sublimation at 80 °C and 0.1 mbar. Further purification was achieved by chromatography on aluminiumoxide (98% dichloromethane/2% isopropanol). Fractions with an R_f value of 0.09 were combined to yield the oily product (3.71 g, 6.6 mmol, 53%). ^{1}H NMR (200 MHz, [D$_4$]MeOH, 25 °C): $\delta = 1.5$ (br m, 2H, $CH_2CH_2CH_2$), 2.12 (s, 12H, pyrazol-CH_3), 2.21 (s, 12H, pyrazol-CH_3), 2.38 (m, 4H, $CH_2CH_2CH_2$), 2.74 (t, $^3J = 6.8$ Hz, 8H, NCH_2CH_2N(pyrazol)), 3.92 (t, $^3J = 6.6$ Hz, 8H, CH_2N(pyrazol)), 5.78 ppm (s, 4H, CH(pyrazol)); ^{13}C NMR (50 MHz, [D$_4$]MeOH, 25 °C): $\delta = 11.23$ (C3(pyrazol)CH_3), 13.03 (C5(pyrazol)CH_3), 25.28 ($CH_2CH_2CH_2$), 48.02 (NCH_2CH_2N(pyrazol)), 53.84 ($CH_2CH_2CH_2$), 55.45 (NCH_2CH_2N-(pyrazol)), 105.99 (CH(pyrazol)), 141.42 (C3(pyrazol)), 148.66 (C5-(pyrazol)); ESI-MS: *m*/*z* (%): 563 (100) [M+H]$^+$; elemental analysis calcd (%) for $C_{31}H_{50}N_{10}$: C 66.16, H 8.95, N 24.89; found: C 65.95, H 8.94, N 24.59.

***N*-Methyl-*N*,*N*-bis-(2-chloroethyl)-ammonium chloride (8)**: *N*-Methyldiethanolamine (5.4 g, 45 mmol) was added dropwise to a solution containing thionylchloride (11.6 mL, 100 mmol) and toluene (50 mL). The solution was heated under reflux for 3 h at 100 °C. The solvent was removed under vacuum. No analysis was made due to the reactivity of this nitrogen mustard analogue. The crude material was used without further purification. See **WARNING** mentioned above!!!

***N*-Methyl-*N*,*N*-bis-[2-(3,5-dimethylpyrazol-1-yl)ethyl]amine (5)**: A solution of 3,5-dimethylpyrazole (14.28 g, 150 mmol) in dry diethyleneglycol dimethylether (200 mL) was stirred with potassium (6 g, 150 mmol) at 70 °C until the metal had dissolved.[37] To this solution, compound **8** (7.06 g, 45 mmol) was added in one portion. This solution was stirred at 120 °C for 4 d. The solvent was removed under vacuum and the residue was purified by chromatography on aluminium oxide (1.5% isopropanol/98.5% dichloromethane). The fractions with an R_f value of 0.24 were combined to yield the product (8.32 g, 30 mmol, 67%). ^{1}H NMR (200 MHz, [D$_4$]MeOH, 25 °C): $\delta = 2.13$ (s, 6H, CH_3), 2.18 (s, 6H, CH_3), 2.29 (s, 3H, NCH_3), 2.72 (t, $^3J = 6.7$ Hz, 4H, CH_2), 3.94 (t, $^3J = 6.7$ Hz, 4H, CH_2), 5.77 ppm (s, 2H, CH); ^{13}C NMR (50 MHz, [D$_4$]MeOH, 25 °C): $\delta = 11.04$ (C3(pyrazol)CH_3), 13.28 (C5(pyrazol)CH_3), 42.93 (NCH_2CH_2N-(pyrazol)), 47.65 (NCH_2CH_2N(pyrazol)), 58.68 (NCH_3), 105.99 (C4-(pyrazol)), 141.47 (C3(pyrazol)), 148.64 ppm (C5(pyrazol)); ESI-MS: *m*/*z* (%): 276 (100) [M+H]$^+$; GC-MS: $t_R = 18.43$ min; 109 (38) [$CH_2-C_5H_7N_2$]$^+$, 123 (20) [$(CH_2)_2-C_5H_7N_2$]$^+$, 166 (100) [$M-$[$(CH_2)_2-C_5H_7N_2$]]$^+$; elemental analysis calcd (%) for $C_{15}H_{25}N_5$: C 65.42, H 9.15, N 25.43; found: C 65.32, H 9.32, N 25.54.

***N,N,N′,N′*-Tetrakis-[2-(3,5-dimethylpyrazol-1-yl)ethyl]-1,3-propylenediamine-bis(nickel(II)-dinitrate) (2)**: Compound **4** (100 mg, 0.18 mmol) was added to a solution containing $Ni(NO_3)_2$·$6H_2O$ (105 mg, 0.36 mmol) and trimethyl orthoformate (27 mmol, 2.92 mL) in methanol (1 mL). This mixture was equilibrated versus diethyl ether by vapour diffusion.[18] After one week, diethyl ether was removed and hexane was added into the outer glass. This mixture was again equilibrated, the precipitated crystals were dissolved in other solvents to grow single crystals. Blue crystals were grown in an acetonitrile solution versus tetrahydrofuran, single crystals suitable for X-ray analysis by slow vapour diffusion of a methanol solution versus tetrahydropyran. ESI-MS: *m*/*z* (%): 292 (100) [ligand+2H]$^{2+}$, 583 (90) [ligand+H]$^+$, 816 (15) [ligand+2Ni+$(NO_3)_2$+H_2O]$^+$; elemental analysis calcd (%) for

$C_{31}H_{50}N_{14}O_{12}Ni_2 \cdot H_2O \cdot 2\,THF$: C 42.96, H 6.29, N 17.98; found: C 42.98, H 6.55, N 18.36.

***N*-Methyl-*N*,*N*-bis-[2-(3,5-dimethylpyrazol-1-yl)ethyl]amine-nickel(II) nitrate (3)**: Compound **5** (100 mg, 0.36 mmol) was added to a solution of $Ni(NO_3)_2 \cdot 6\,H_2O$ (105 mg, 0.36 mmol) and trimethyl orthoformate[18] (2.92 mL, 27 mmol) in methanol (1 mL). Blue crystals were grown in an acetonitrile solution versus tetrahydrofuran, single crystals suitable for X-ray analysis by slow vapour diffusion of a methanol solution versus tetrahydropyran. ESI-MS: *m*/*z* (%): 276 (100) $[ligand+H]^+$, 446 (27) $[ligand+Ni+NO_3+CH_3OH+H_2O]^+$; elemental analysis calcd (%) for $C_{15}H_{25}N_7NiO_6$: C 39.33, H 5.50, N 21.40; found: C 39.70, H 5.09, N 21.28.

CD measurements: CD titrations of poly d(GC) with **2**, **3**, 1:1 adduct of **4** and $Ni(NO_3)_2$, **4**, and **5** were performed as follows: A 0.1 mM poly d(GC) solution in 1 mM sodium cacodylate buffer (pH 7) was titrated with various aliquots of 1 mM solutions of the metal complexes **2**, **3**, the 1:1 adduct of $Ni(NO_3)_2$ and **4** as well as ligands **4** and **5** in the same buffer. The samples were warmed to 60 °C for 5 min and then cooled down to 25 °C for the CD measurements. The CD spectra were smoothed by adjacent averaging.

Binding constants determination: The proton and nickel binding constants of **4** and **5** were determined as follows: The pH titrations were carried out on a Metrohm potentiograph E 356 at 25 °C in 0.1 M $NaNO_3$ containing 40% isopropanol for **4** and 40% methanol for **5**. The titration curves were analysed with the program Hyperquad2006.[38,39] The logarithmic values of the ionic product of water (pK_W) in the presence of the organic cosolvents were taken from the literature[40,41] and corrected for the ionic strength using Specific Interaction Theory (SIT).[42] The determination of metal binding constants was performed analogously in the presence of two (in case of **4**) or one (in case of **5**) equivalents of nickel.

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