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# Ring-functionalized niobocene complexes

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**A series of new ring-functionalized niobocene dichloride compounds $(RCH_2C_5H_4)_2NbCl_2$ (R = $MeOCH_2$, $(CH_2)_5NCH_2$, 2-$MeOC_6H_4$, 3-$MeOC_6H_4$, 4-$MeOC_6H_4$, 2,4-$(MeO)_2C_6H_3$, 3,4-$(MeO)_2C_6H_3$, 2,4,6-$(MeO)_3C_6H_2$, 3,4,5-$(MeO)_3C_6H_2$, 4-$FC_6H_4$, 4-$Me_2NC_6H_4$) was synthesized and characterized by electron paramagnetic resonance. Three structures were determined by X-ray diffraction analysis and revealed the expected bent metallocene structure with two cyclopentadienyl ligands and two chlorides coordinated to niobium in the oxidation state IV. Chlorides and the centroids of the $\eta^5$-bonded cyclopentadienyl rings define a distorted tetrahedron. The cytotoxicity study has demonstrated that substitution with methoxybenzyl groups can lead to highly active species, but the activity strongly varies with the number and positions of the methoxy groups in the benzene ring. The most active species under study – $\{2,4\text{-}(MeO)_2C_6H_3CH_2C_5H_4\}_2NbCl_2$ – has a maximal inhibitory concentration value comparable with cisplatin. Copyright © 2014 John Wiley & Sons, Ltd.**

**Keywords:** metallocene dihalides; cytotoxicity; EPR spectroscopy; X-ray diffraction analysis; leukemia therapy

## Introduction

Metallocene dichloride complexes $Cp_2MCl_2$ (Cp = $\eta^5$-$C_5H_5$; M = group 4–6 metal) are under comprehensive scrutiny since the cytostatic activity of $Cp_2TiCl_2$ was discovered in 1979.[1] The early stage of the investigation has shown that metallocene compounds of titanium, vanadium, niobium and molybdenum are highly active toward various tumor cell lines, whereas compounds of the other group 4–6 metals have considerably lower activity or are inactive.[2] In the last decade, several approaches were utilized for modification of metallocene compounds that led to improvement of their properties relevant to biological applications.[3,4] Metallocene complexes were encapsulated into the cavity of the cyclodextrin molecule, which considerably enhanced their water solubility.[5–8] A similar effect can be gained by exchange of chloride ligands.[9–11] Nevertheless, the exchange of chloride ligands usually does not improve cytotoxicity much because the putative active metallocene species $[Cp_2M]^{2+}$ is not changed.[12] One of few examples, where this approach leads to highly cytotoxic compounds, was published recently. Vanadocene complex bearing 5-amino-1,10-phenanthroline $[Cp_2V(5\text{-}NH_2\text{-phen})][OTf]_2$ is about 20 times more active toward leukemia cells (MOLT-4) than the parent dichloride species ($Cp_2VCl_2$) as a result of the higher stability in aqueous media.[13] Another promising approach for design of the highly active metallocene species involves modification of the $[Cp_2M]^{2+}$ moiety through substitution in the cyclopentadienyl rings. Hence various metallocene species bearing ester, aminoalkyl, aminobenzyl and methoxybenzyl groups in the cyclopentadienyl rings were found to be active toward several cisplatin-resistant tumor cell lines.[14–25]

This work follows our recent study on ring-functionalized niobocene compounds. We have observed that parent ($Cp_2NbCl_2$) exhibits only medium activity toward MOLT-4 and highly active niobocene species are yet to be generated, since substitution with ester groups led to minor improvements only.[16] The aim of this work is to evaluate methoxyethyl-, methoxybenzyl-, aminoalkyl-, aminobenzyl- and fluorobenzyl- substituents in an attempt to find highly cytotoxic niobocene complexes that might be suitable for future biological investigation.

## Results and Discussion

### Preparation and Characterization of Niobocene Compounds

Ring-substituted niobocene dichlorides were synthesized from appropriately substituted cyclopentadienes or fulvenes (Schemes 1 and 2). The starting cyclopentadienes **1** and **2** were prepared from sodium cyclopentadienide and functionalized ethyl chloride according to published procedures.[26,27] The condensation of substituted benzaldehyde with cyclopentadiene in the presence of pyrrolidine as base was used for synthesis of starting fulvenes **3–11**.[28] Deprotonation of cyclopentadienes **1** and **2** with *n*-BuLi followed by addition of $NbCl_4(THF)_2$ produces niobocene dichloride complexes $(MeOCH_2CH_2C_5H_4)_2NbCl_2$ (**12**) and $\{(CH_2)_5NCH_2CH_2C_5H_4\}_2NbCl_2$ (**13**), respectively (Scheme 1).

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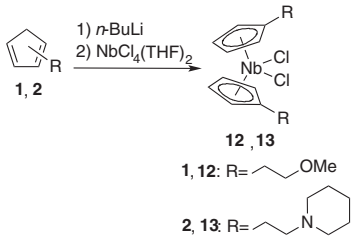
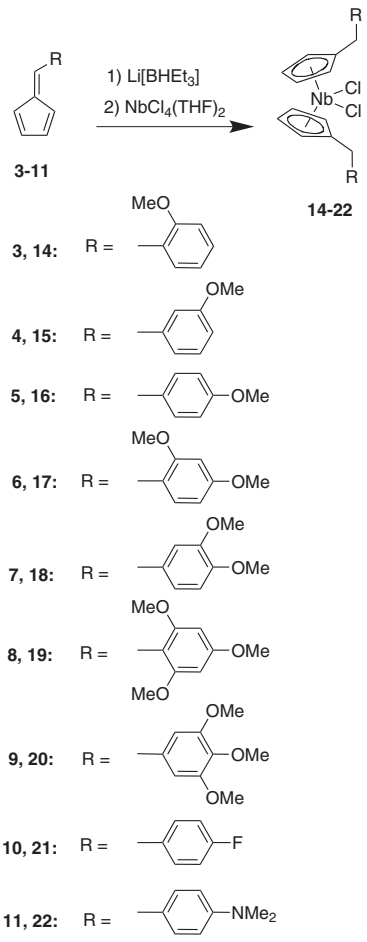



**Scheme 1.** Synthesis of niobocene dichlorides **12** and **13**.

The reaction of $NbCl_4(THF)_2$ with substituted lithium cyclopentadienide was also used for synthesis of benzyl-substituted niobocene dichloride complexes $(RCH_2C_5H_4)_2NbCl_2$ (**14–22**) (see Scheme 2). The starting benzyl-substituted cyclopentadienides were prepared *in situ* by hydrolithiation of phenyl-substituted fulvenes **3–11**.

All reported niobocene complexes (**12–22**) were characterized by electron paramagnetic resonance (EPR) spectroscopy. These spectra were measured in dichloromethane and show the expected ten-line hyperfine coupling (HFC) corresponding to the nuclear spin value of $^{93}Nb$ ($I = 9/2$, 100%; see Fig. 1). Isotropic HFC constants and isotropic *g*-factors of compounds **12–22** are listed in Table 1. The reported ring-functionalized niobocene




**Scheme 2.** Synthesis of niobocene dichlorides **14–22**.

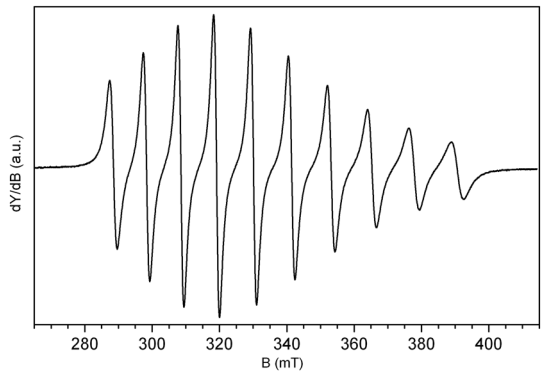



**Figure 1.** EPR spectrum of compound **17** in $CH_2Cl_2$ ($\nu = 9.4172$ GHz).

**Table 1.** Isotropic HFC constants ($10^{-4}$ $cm^{-1}$) and isotropic *g*-factors of niobocene complexes

| | $\lvert A_{iso}\rvert$ | $g_{iso}$ |
|---|---|---|
| $Cp_2NbCl_2$[a] | 107.4 | 1.979 |
| **12** | 104.8 | 1.980 |
| **13** | 105.6 | 1.980 |
| **14** | 105.0 | 1.981 |
| **15** | 105.5 | 1.981 |
| **16** | 105.5 | 1.981 |
| **17** | 105.1 | 1.981 |
| **18** | 105.4 | 1.982 |
| **19** | 105.3 | 1.981 |
| **20** | 105.2 | 1.981 |
| **21** | 105.9 | 1.981 |
| **22** | 105.5 | 1.980 |

[a]Data reported in literature.[16]

dichlorides show only slightly lower values of isotropic HFC constants ($|A_{iso}| = 104.8–105.9 \times 10^{-4}$ $cm^{-1}$) and slightly higher isotropic *g*-factors ($g_{iso} = 1.980–1.982$) than observed for unsubstituted niobocene dichloride ($|A_{iso}| = 107.4 \times 10^{-4}$ $cm^{-1}$, $g_{iso} = 1.979$).[16] This reveals that this substitution pattern has only negligible effect on delocalization of the unpaired electron that occupies the antibonding orbital of the Nb—Cl bonds.[29]

### X-Ray Structures of Compounds 12, 14 and 17

Structures of **12**, **14** and **17** were determined by single-crystal X-ray diffraction analysis. Molecular structures of these complexes are shown in Figs. 2–4, and selected bond lengths and bond angles describing the coordination sphere of the central metal are summarized in Table 2. The molecules of **12** and **14** are $C_s$ symmetric, whereas **17** belongs to the $C_2$ point group. Complexes **12**, **14** and **17** have typical bent metallocene structure with two cyclopentadienyl ligands and two chlorides coordinated to niobium in the oxidation state IV. Chlorides and the centroids of the $\eta^5$-bonded cyclopentadienyl rings make a distorted tetrahedron around central metal. The bond distances Nb—Cg(Cp) and Nb—Cl are in the narrow range 2.081(2)–2.094(2) Å and 2.456(2)–2.473(2) Å, respectively. Bond angles Cg(Cp)—Nb—Cg(Cp) and Cl—Nb—Cl were found to be in range 131.8(1)–132.6(1)°

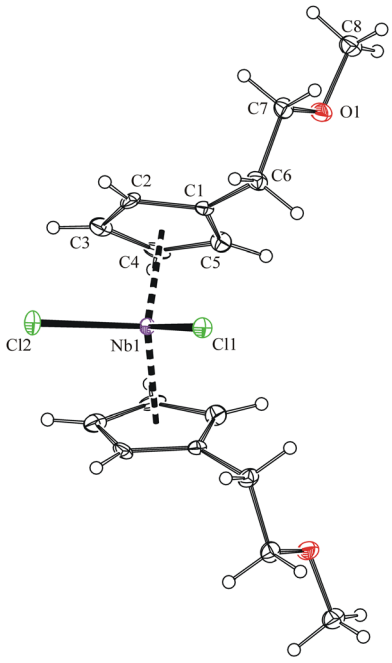



**Figure 2.** Molecular structure of $(MeOCH_2CH_2C_5H_4)_2NbCl_2$ (**12**) showing the labeling scheme for all non-hydrogen atoms. Anisotropic displacement ellipsoids are drawn at the 30% probability level. Unlabeled atoms are related by symmetry operation: $-x$; $-y$, $½ + z$.

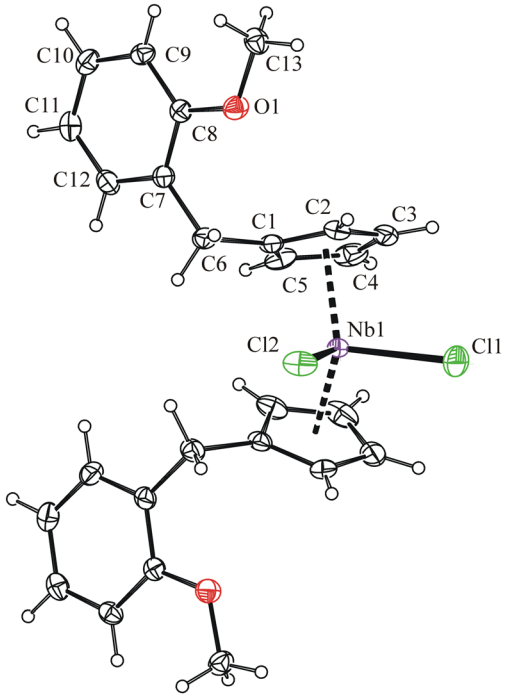



**Figure 3.** Molecular structure of $(2\text{-}MeOC_6H_4CH_2C_5H_4)_2NbCl_2$ (**14**) showing the labeling scheme for all non-hydrogen atoms. Anisotropic displacement ellipsoids are drawn at the 30% probability level. Unlabeled atoms are related by symmetry operation: $½ - x$; $-y$, $½ + z$.

and 86.0(1)–86.8(1)°, respectively. The reported geometric parameters for the substituted niobocene species are in line with values previously reported for unsubstituted counterpart $Cp_2NbCl_2$.[30]

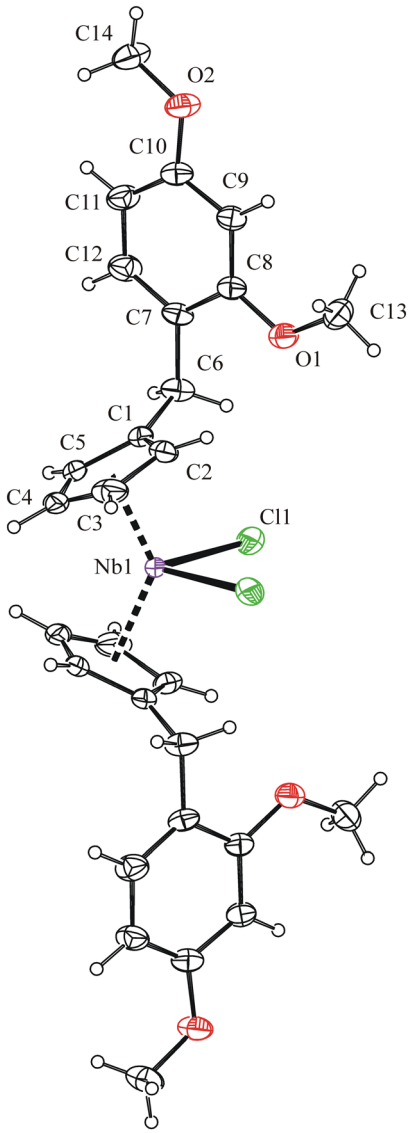



**Figure 4.** Molecular structure of $\{2,4\text{-}(MeO)_2C_6H_3CH_2C_5H_4\}_2NbCl_2$ (**17**), showing the labeling scheme for all non-hydrogen atoms. Anisotropic displacement ellipsoids are drawn at the 30% probability level. Unlabeled atoms are related by symmetry operation: $-x$; $y$, $½ - z$.

## Cytotoxicity Studies of Niobocene Complexes

Cytotoxic effects of **12–22** were evaluated on human T-lymphocytic leukemia cells MOLT-4 in exponential growth phase, 24 h after the incubation with the cytostatic drugs. Table 3 summarizes the half-maximal inhibitory concentration values ($IC_{50}$) obtained from the standard WST-1 viability assays.[31] The compounds under study show solubility in water. Nevertheless, standard protocol including dissolution in DMSO followed by dilution with cultivation medium enabled examination of cytotoxicity up to a concentration of 150 μM.

The cytotoxicity of the niobocene compounds under study varies in a wide range from highly active species to almost inactive. Medium to high activity was observed in the case of methoxybenzyl-substituted compounds **16**, **17** and **20** ($IC_{50}$ = 14–46 μM; see Fig. 5). These species have similar or higher activity than unsubstituted niobocene dichloride. Compounds **13–15** show considerably lower cytotoxicity ($IC_{50}$ = 59–80 μM) and

**Table 2.** Structural parameters (Å, °) of niobocene compounds, describing the coordination around the metal

| | Nb—$Cg_1$ | Nb—$Cg_2$ | Nb—$Cl_1$ | Nb—$Cl_2$ | $Cg_1$—Nb—$Cg_2$ | $Cl_1$—Nb—$Cl_2$ |
|---|---|---|---|---|---|---|
| $Cp_2NbCl_2$[a] | 2.085 | 2.090 | 2.468(4) | 2.476(3) | 131.6 | 85.5(0.1) |
| | 2.092 | 2.087 | 2.464(5) | 2.475(4) | 132.1 | 85.7(0.2) |
| **12** | 2.0903(19) | 2.0906(19) | 2.4614(12) | 2.4732(14) | 132.48(8) | 86.04(5) |
| **14** | 2.0814(16) | 2.0815(16) | 2.4689(15) | 2.4658(15) | 131.84(7) | 86.12(5) |
| **17** | 2.094(2) | 2.094(2) | 2.4562(18) | 2.4562(18) | 132.62(10) | 86.82(6) |

[a]Data reported in the literature.[30]

**Table 3.** Cytotoxicity of the metallocene complexes toward MOLT-4 cells expressed as the $IC_{50}$ values (μM)

| | $IC_{50}$ | | $IC_{50}$ |
|---|---|---|---|
| $Cp_2NbCl_2$[a] | 49 ± 5 | **18** | >150 |
| **12** | >150 | **19** | >150 |
| **13** | 63 ± 4 | **20** | 32 ± 2 |
| **14** | 80 ± 5 | **21** | >150 |
| **15** | 59 ± 8 | **22** | >150 |
| **16** | 46 ± 4 | $Cp_2VCl_2$[b] | 70 ± 7 |
| **17** | 14 ± 1 | $(4\text{-}MeOC_6H_4CH_2C_5H_4)_2VCl_2$[b] | 11 ± 7 |

[a]Data reported in the literature.[16]
[b]Data reported in the literature.[22]

compounds **12**, **18**, **19**, **21** and **22** are almost inactive ($IC_{50} > 150$ μM). Differences in activity of the ring-substituted niobocene compounds reflect the modification of putative active niobocene species $[Cp'_2Nb]^{2+}$, although there is no simple relation between the structure of the niobocene compound and its cytotoxicity; in particular, substitution patterns bearing methoxy groups in the benzyl substituent seem to be very effective. One could expect high cytotoxicity for 4-methoxybenzyl-substituted compound **16**, based on literature data published for related titanium,[4] vanadium[22,24] and molybdenum complexes[25] (e.g. data reported for vanadocene analogues; see Table 3). However, this substitution pattern brings only minor improvement for niobocene dichloride. A considerable increase in cytotoxicity is observed for 2,4-dimethoxybenzyl-substituted compound **17**.

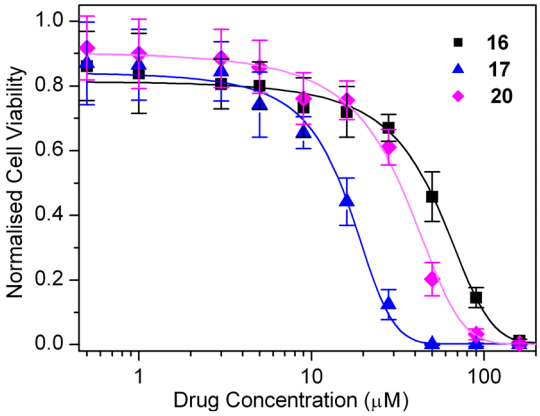



**Figure 5.** Cytotoxicity curves showing the effect of the compounds **16**, **17** and **20** on the viability of the leukemia cells MOLT-4.

Hence this compound is about three times more active than unsubstituted niobocene dichloride.

## Conclusions

This study demonstrates that the standard protocol for niobocene dichloride, starting from solvate of niobium tetrachloride, is compatible with the presence of various functional groups in the cyclopentadienyl ring. The newly synthesized derivatives cover several substitution patterns as previously successfully used for design of the highly cytotoxic titanocene, vanadocene and molybdenocene species. The cytotoxicity study proves that substitution with methoxybenzyl groups can lead to highly active species. The detailed study on various methoxybenzyl compounds has shown that their activity strongly varies with number and position of the methoxy groups in the benzene ring. The highest cytotoxic effect was detected at the derivative bearing 2,4-dimethoxybenzyl groups (**17**). The $IC_{50}$ value of this compound was found to be about three times lower than observed for unsubstituted niobocene dichloride and comparable with cisplatin (15.8 ± 1.9 μM).[13]

## Experimental

### Methods and Materials

All operations were performed under nitrogen using conventional Schlenk-line techniques. The solvents were purified and dried by standard methods.[32] Methoxyethylcyclopentadiene (**1**),[26] piperidinoethylcyclopentadine (**2**),[27] 6-(2′-methoxyphenyl)fulvene (**3**),[22] 6-(3′-methoxyphenyl)fulvene (**4**),[33] 6-(4′-methoxyphenyl)fulvene (**5**),[34] 6-(2′,4′-dimethoxyphenyl)fulvene (**6**),[35] 6-(3′,4′-dimethoxyphenyl)fulvene (**7**),[33] 6-(2′,4′,6′-trimethoxyphenyl)fulvene (**8**),[34] 6-(3′,4′,5′-trimethoxyphenyl)fulvene (**9**),[33] 6-(4′-dimethylaminophenyl)fulvene (**11**)[36] and $NbCl_4(THF)_2$[37] were prepared according to literature procedures. The other starting materials were available commercially (Sigma-Aldrich or Acros Organics). EPR spectra were recorded on Miniscope MS 300 spectrometers at X-band at ambient temperature.

### Synthesis of Ligand Precursor

*Preparation of 6-(4′-fluorophenyl)fulvene (**10**)*

Pyrrolidine (9 ml, 110 mmol) was added dropwise to the mixture of freshly monomerized cyclopentadiene (15 ml, 179 mmol) and 4-fluorobenzaldehyde (10.3 g, 69 mmol) in methanol (150 ml). After addition, the solution was stirred at room temperature for 1 h and then acetic acid (6 ml, 105 mmol) was added. The reaction mixture was treated with water. The dark-red solid was

**Table 4.** Crystallographic data for niobocene compounds **12**, **14** and **17**

| Compound | **12** | **14** | **17** |
|---|---|---|---|
| Formula | $C_{16}H_{22}Cl_2NbO_2$ | $C_{26}H_{26}Cl_2NbO_2$ | $C_{28}H_{30}Cl_2NbO_4$ |
| Mol. wt | 410.15 | 534.28 | 594.33 |
| Cryst. syst. | Orthorhombic | Orthorhombic | Monoclinic |
| Space group | $Cmc2_1$ (No. 36) | *Pnma* (No. 62) | *C*2/*c* (No. 15) |
| *a* (Å) | 19.4502(6) | 14.5240(7) | 27.8663(6) |
| *b* (Å) | 7.2050(3) | 21.9711(9) | 6.7130(2) |
| *c* (Å) | 11.5434(6) | 6.9961(17) | 17.2082(4) |
| $\beta$ (°) | 90 | 90 | 125.331(3) |
| *Z* | 4 | 4 | 4 |
| $\mu$ ($mm^{-1}$) | 1.075 | 0.800 | 0.694 |
| $D_x$ (g $cm^{-3}$) | 1.684 | 1.590 | 1.503 |
| Crystal size (mm) | $0.17\times0.19\times0.25$ | $0.19\times0.21\times0.29$ | $0.19\times0.26\times0.33$ |
| $\theta$ range (°) | 3.0–27.5 | 1.9–29.5 | 2.4–27.5 |
| No. of reflections measured | 6602 | 18 661 | 8333 |
| No. of unique reflections; $R_{int}$ | 1840, 0.090 | 3151, 0.092 | 2969, 0.038 |
| No. of observed reflections [$I > 2\sigma(I)$] | 1782 | 2402 | 2516 |
| No. of parameters | 100 | 145 | 159 |
| [b]All data | 1.14 | 1.10 | 1.25 |
| *R*, $wR^2$ | 0.038, 0.089 | 0.046, 0.100 | 0.057, 0.132 |
| $\Delta\rho$, max., min. (e $Å^{-3}$) | 0.51, −0.73 | 0.62, −0.63 | 0.96, −0.44 |

filtered off and purified by Soxhlet extraction with pentane. Yield 10.2 g (59 mmol, 86%); red powder. Analytical and spectroscopic data are in agreement with literature values.[38]

## Synthesis of Niobocene Complexes

*Preparation of $(MeOCH_2CH_2C_5H_4)_2NbCl_2$ (**12**)*

Methoxyethylcyclopentadiene (**1**; 0.29 g, 2.34 mmol) was dissolved in THF (10 ml), treated dropwise with 1.6 M solution of *n*-BuLi (1.50 ml, 2.40 mmol) in hexane and stirred overnight. The solution of substituted cyclopentadienide was added to the suspension of $NbCl_4(THF)_2$ (0.42 g, 1.12 mmol) in THF (10 ml) pre-cooled at 0°C. The reaction mixture was stirred overnight. The volatiles were evaporated under vacuum. The crude product was dissolved in dichloromethane (10 ml) and filtered over a thin pad of celite. The filtrate was treated with hexane (20 ml) and the formed precipitate was decanted, washed with diethyl ether ($3\times10$ ml) and vacuum dried. Yield 0.33 g (0.80 mmol, 71%); brown powder. Anal. Calcd for $C_{16}H_{22}Cl_2NbO_2$: C 46.85; H 5.41. Found: C 46.69; H 5.28. EPR($CH_2Cl_2$): $g_{iso} = 1.980$, $|A_{iso}| = 113.4$ G. Single crystals of **12** suitable for X-ray analysis were obtained by careful layering of the $CH_2Cl_2$ solution with a double volume of hexane.

*Preparation of $\{(CH_2)_5NCH_2CH_2C_5H_4\}_2NbCl_2$ (**13**)*

The steps of synthesis followed the procedure for compound **12**. Reagents: $NbCl_4(THF)_2$ (0.72 g, 1.90 mmol), piperidinoethylcyclopentadine (**2**; 0.68 g, 3.84 mmol) and *n*-BuLi (2.5 ml, 4.00 mmol). Yield: 0.61 g (1.18 mmol, 62%); brown powder. Anal. Calcd for $C_{24}H_{36}Cl_2N_2Nb$: C 55.83; H 7.03; N 5.43. Found: C 55.67; H 7.19; N 5.51. EPR($CH_2Cl_2$): $g_{iso} = 1.980$, $|A_{iso}| = 114.3$ G.

*Preparation of $(2\text{-}MeOC_6H_4CH_2C_5H_4)_2NbCl_2$ (**14**)*

A Schlenk flask was treated with a 1 M solution of Super-Hydride (4.60 ml, 4.60 mmol) in THF and solvent was removed under vacuum at 90°C during 1 h. 6-(2′-Methoxyphenyl)fulvene (**3**; 0.84 g, 4.57 mmol) was dissolved in diethyl ether (10 ml) added to the solution of Super-Hydride in diethyl ether. This mixture was stirred overnight. The white precipitate was decanted, washed with diethyl ether ($3\times5$ ml) and vacuum dried. The white solid was dissolved in THF (10 ml) and added to a suspension of $NbCl_4(THF)_2$ (0.86 g, 2.27 mmol) in THF (10 ml) pre-cooled at 0 °C. The reaction mixture was stirred at room temperature overnight. The solvent was evaporated under vacuum. The crude product was dissolved in dichloromethane (10 ml) and filtered over short pad of celite. The filtrate was treated with hexane (20 ml) and the formed precipitate was decanted, washed with diethyl ether ($3\times10$ ml) and vacuum dried. Yield 0.68 g (1.27 mmol, 56%); brown powder. Anal. Calcd for $C_{26}H_{26}Cl_2NbO_2$: C 58.45; H 4.90. Found: C 58.68; H 5.01. EPR($CH_2Cl_2$): $g_{iso} = 1.981$, $|A_{iso}| = 113.5$ G. Single crystals of **14** suitable for X-ray analysis were obtained by careful layering of the $CH_2Cl_2$ solution with a double volume of hexane.

*Preparation of $(3\text{-}MeOC_6H_4CH_2C_5H_4)_2NbCl_2$ (**15**)*

The steps of synthesis followed the procedure for compound **14**. Reagents: $NbCl_4(THF)_2$ (0.30 g, 0.79 mmol), 6-(3′-methoxyphenyl) fulvene (**4**; 0.32 g, 1.74 mmol) and Super-Hydride (1.70 ml, 1.70 mmol). Yield 0.26 g (0.49 mmol, 62%); brown powder. Anal. Calcd for $C_{26}H_{26}Cl_2NbO_2$: C 58.45; H: 4.90. Found: C 58.54; H: 4.71. EPR ($CH_2Cl_2$): $g_{iso} = 1.981$, $|A_{iso}| = 114.1$ G.

*Preparation of $(4\text{-}MeOC_6H_4CH_2C_5H_4)_2NbCl_2$ (**16**)*

The steps of synthesis followed the procedure for compound **14**. Reagents: $NbCl_4(THF)_2$ (0.64 g, 1.69 mmol), 6-(4′-methoxyphenyl) fulvene (**5**; 0.63 g, 3.42 mmol) and Super-Hydride (3.45 ml, 3.45 mmol). Yield 0.58 g (1.09 mmol, 64%); brown powder. Anal. Calcd for $C_{26}H_{26}Cl_2NbO_2$: C 58.45; H: 4.90. Found: C 58.72; H: 4.69. EPR ($CH_2Cl_2$): $g_{iso} = 1.981$, $|A_{iso}| = 114.1$ G.

*Preparation of $\{2,4\text{-}(MeO)_2C_6H_3CH_2C_5H_4\}_2NbCl_2$ (**17**)*

The steps of synthesis followed the procedure for compound **14**. Reagents: $NbCl_4(THF)_2$ (0.52 g, 1.37 mmol), 6-(2′,4′-dimethoxyphenyl)

fulvene (**6**; 0.59 g, 2.76 mmol) and Super-Hydride (2.85 ml, 2.85 mmol). Yield 0.48 g (0.81 mmol, 59%); brown powder. Anal. Calcd for $C_{28}H_{30}Cl_2NbO_4$: C 56.58; H: 5.09. Found: C 56.52; H 5.01. EPR ($CH_2Cl_2$): $g_{iso} = 1.981$, $|A_{iso}| = 113.6$ G. Single crystals of **17** suitable for X-ray analysis were obtained by careful layering of the $CH_2Cl_2$ solution with a double volume of hexane.

*Preparation of {3,4-(MeO)$_2$C$_6$H$_3$CH$_2$C$_5$H$_4$}$_2$NbCl$_2$ (**18**)*

The steps of synthesis followed the procedure for compound **14**. Reagents: $NbCl_4(THF)_2$ (0.63 g, 1.66 mmol), 6-(3′,4′-dimethoxyphenyl)fulvene (**7**; 0.76 g, 3.55 mmol) and Super-Hydride (3.50 ml, 3.50 mmol). Yield 0.52 g (0.88 mmol, 53%); brown powder. Anal. Calcd for $C_{28}H_{30}Cl_2NbO_4$: C 56.58; H: 5.09. Found: C 56.32; H: 4.92. EPR($CH_2Cl_2$): $g_{iso} = 1.982$, $|A_{iso}| = 113.9$ G.

*Preparation of {2,4,6-(MeO)$_3$C$_6$H$_2$CH$_2$C$_5$H$_4$}$_2$NbCl$_2$ (**19**)*

The steps of synthesis followed the procedure for compound **14**. Reagents: $NbCl_4(THF)_2$ (0.76 g, 2.01 mmol), 6-(2′,4′,6′-trimethoxyphenyl)fulvene (**8**; 0.98 g, 4.02 mmol) and Super-Hydride (4.00 ml, 4.00 mmol). Yield 0.64 g (0.98 mmol, 49%); brown powder. Anal. Calcd for $C_{30}H_{34}Cl_2NbO_6$: C: 55.06; H: 5.24. Found: C: 55.14; H: 5.33. EPR($CH_2Cl_2$): $g_{iso} = 1.981$, $|A_{iso}| = 113.9$ G.

*Preparation of {3,4,5-(MeO)$_3$C$_6$H$_2$CH$_2$C$_5$H$_4$}$_2$NbCl$_2$ (**20**)*

The steps of synthesis followed the procedure for compound **14**. Reagents: $NbCl_4(THF)_2$ (0.65 g, 1.72 mmol), 6-(3′,4′,5′-trimethoxyphenyl)fulvene (**9**; 0.84 g, 3.44 mmol) and Super-Hydride (3.43 ml, 3.43 mmol). Yield 0.58 g (0.89 mmol, 52%); brown powder. Anal. Calcd for $C_{30}H_{34}Cl_2NbO_6$: C 55.06; H 5.24. Found: C 54.88; H 5.42. EPR($CH_2Cl_2$): $g_{iso} = 1.981$, $|A_{iso}| = 113.8$ G.

*Preparation of (4-FC$_6$H$_4$CH$_2$C$_5$H$_4$)$_2$NbCl$_2$ (**21**)*

The steps of synthesis followed the procedure for compound **14**. Reagents: $NbCl_4(THF)_2$ (0.56 g, 1.40 mmol), 6-(4′-fluorophenyl) fulvene (**10**; 0.53 g, 3.08 mmol) and Super-Hydride (3.10 ml, 3.10 mmol). Yield 0.47 g (0.92 mmol, 66%); brown powder. Anal. Calcd for $C_{24}H_{20}Cl_2F_2Nb$: C 56.50; H 3.95. Found: C 56.62; H 4.03. EPR($CH_2Cl_2$): $g_{iso} = 1.981$, $|A_{iso}| = 114.5$ G.

*Preparation of (4-Me$_2$NC$_6$H$_4$CH$_2$C$_5$H$_4$)$_2$NbCl$_2$ (**22**)*

The steps of synthesis followed the procedure for compound **14**. Reagents: $NbCl_4(THF)_2$ (0.59 g, 1.56 mmol), 6-(4′-dimethylaminophenyl)fulvene (**11**; 0.62 g, 3.16 mmol) and Super-Hydride (3.20 ml, 3.20 mmol). Yield: 0.50 g (0.90 mmol, 58%). Brown powder. Anal. Calcd for $C_{28}H_{32}Cl_2N_2Nb$: C 60.01; H: 5.76; N 5.00. Found: C 60.12; H 5.53; N 5.06. EPR($CH_2Cl_2$): $g_{iso} = 1.980$, $|A_{iso}| = 114.1$ G.

### Cytotoxicity Studies

Studies were performed on human T-lymphocytic leukemia cells MOLT-4 obtained from the American Type Culture Collection (USA). The cells were cultured in Iscove's modified Dulbecco's medium supplemented with a 20% fetal calf serum and 0.05% L-glutamine (all Sigma-Aldrich, USA) in a humidified incubator at 37°C and a controlled 5% $CO_2$ atmosphere. The cell lines in the maximal range of up to 20 passages have been used for this study.

Cytotoxicity of compounds **12–22** was evaluated by the WST-1 cell viability test (Roche, Germany) according to manufacturer's instructions. The assay is based on the reduction of WST-1 (4-[3-(4-Iodophenyl)-2-(4-nitrophenyl)-2*H*-5-tetrazolio]-1,3-benzene disulfonate) by viable cells. The reaction produces a colored soluble formazan salt. Absorbance at 440 nm was measured using a multiplate reader (Tecan Infinite 200). Compounds **12–22** were dissolved in DMSO and diluted by cultivation medium to desired concentrations. The MOLT-4 cells were seeded in a 96-well plate, incubated in solutions of compounds **12–22** for 24 h, then washed in pure media and incubated for 180 min in WST-1 solution. The same cells incubated in the cultivation media only were used as the control.

### Crystallography

The X-ray data for crystals of **12**, **14** and **17** were obtained at 150 K using an Oxford Cryostream low-temperature device on a Nonius KappaCCD diffractometer with Mo-$K_\alpha$ radiation ($\lambda = 0.71073$ Å), a graphite monochromator, and the $\phi$ and $\chi$ scan mode. Data reductions were performed with DENZO-SMN.[39] Absorption was corrected by integration methods.[40] Structures were solved by direct methods (SIR92)[41] and refined by full-matrix least squares based on $F^2$ (SHELXL97).[42] Hydrogen atoms were mostly localized on a difference Fourier map; however, to ensure uniformity of treatment of crystal, all hydrogens were recalculated into idealized positions (riding model) and assigned temperature factors $H_{iso}(H) = 1.2\ U_{eq}$ (pivot atom) or $1.5\ U_{eq}$ (methyl). The absolute structure of **12** was determined according to standard Flack procedures.[43] Crystallographic data are summarized in Table 4. Figs. 2–4 were drawn using PLATON.[44]

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## References

[1] H. Köpf, P. Köpf-Maier, *Angew. Chem. Int. Edit.* **1979**, *18*, 477–478.
[2] P. Köpf-Maier, H. Köpf, *Struct. Bonding* **1988**, 103–194.
[3] P. M. Abeysinghe, M. M. Harding, *Dalton Trans.* **2007**, 3474–3482.
[4] K. Strohfeldt, M. Tacke, *Chem. Soc. Rev.* **2008**, *37*, 1174–1187.
[5] C. C. L. Pereira, M. Nolasco, S. S. Braga, F. A. A. Paz, P. Ribeiro-Claro, M. Pillinger, I. S. Gonçalves, *Organometallics* **2007**, *26*, 4220–4228.
[6] I. Turel, A. Demsar, J. Kosmrlj, *J. Incl. Phenom. Macrocycl. Chem.* **1999**, *35*, 595–604.
[7] J. Vinklárek, J. Honzíček, J. Holubová, *Cent. Eur. J. Chem.* **2005**, *3*, 72–81.
[8] S. S. Braga, I. S. Gonçalves, M. Pillinger, P. Ribeiro-Claro, J. J. C. Teixeira-Dias, *J. Organomet. Chem.* **2001**, *632*, 11–16.
[9] P. Köpf-Maier, H. Köpf, *Chem. Rev.* **1987**, *87*, 1137–1152.
[10] J. Vinklárek, H. Paláčková, J. Honzíček, *Collect. Czech. Chem. Commun.* **2004**, *69*, 811–821.
[11] P. Köpf-Maier, I. C. Tornieporth-Oetting, *Biometals* **1996**, *9*, 267–271.
[12] J. Vinklárek, H. Paláčková, J. Honzíček, J. Holubová, M. Holčapek, I. Císařová, *Inorg. Chem.* **2006**, *45*, 2156–2162.
[13] J. Vinklárek, H. Hurychová, J. Honzíček, L. Šebestová, Z. Padělková, M. Řezáčová, *Eur. J. Inorg. Chem.* **2013**, 2665–2672.
[14] O. R. Allen, A. L. Gott, J. A. Hartley, J. M. Hartley, R. J. Knox, P. C. McGowan, *Dalton Trans.* **2007**, 5082–5090.
[15] J. R. Boyles, M. C. Baird, B. G. Campling, N. Jain, *J. Inorg. Biochem.* **2001**, *84*, 159–162.
[16] I. Klepalová, J. Honzíček, J. Vinklárek, Z. Padělková, L. Šebestová, M. Řezáčová, *Inorg. Chim. Acta* **2013**, *402*, 109–115.
[17] J. Ceballos-Torres, S. Gómez-Ruiz, G. N. Kaluđerović, M. Fajardo, R. Paschke, S. Prashar, *J. Organomet. Chem.* **2012**, *700*, 188–193.
[18] W. Cini, T. D. Bradshaw, W. Lewis, S. Woodward, *Eur. J. Org. Chem.* **2013**, 3997–4007.
[19] O. R. Allen, L. Croll, A. L. Gott, R. J. Knox, P. C. McGowan, *Organometallics* **2004**, *23*, 288–292.
[20] N. J. Sweeney, O. Mendoza, H. Müller-Bunz, C. Pampillón, F.-J. K. Rehmann, K. Strohfeldt, M. Tacke, *J. Organomet. Chem.* **2005**, *690*, 4537–4544.

[21] P. W. Causey, M. C. Baird, S. P. C. Cole, *Organometallics* **2004**, *23*, 4486–4494.
[22] J. Honzíček, J. Vinklárek, I. Klepalová, Z. Padělková, I. Císařová, P. Šiman, M. Řezáčová, *Inorg. Chim. Acta* **2011**, *373*, 1–7.
[23] B. Gleeson, J. Claffey, A. Deally, M. Hogan, L. M. M. Méndez, H. Müller-Bunz, S. Patil, D. Wallis, M. Tacke, *Eur. J. Inorg. Chem.* **2009**, 2804–2810.
[24] B. Gleeson, J. Claffey, M. Hogan, H. Müller-Bunz, D. Wallis, M. Tacke, *J. Organomet. Chem.* **2009**, *694*, 1369–1374.
[25] B. Gleeson, J. Claffey, A. Deally, M. Hogan, L. M. M. Méndez, H. Müller-Bunz, S. Patil, M. Tacke, *Inorg. Chim. Acta* **2010**, *363*, 1831–1836.
[26] J. Honzíček, A. Mukhopadhyay, T. S. Silva, M. J. Romão, C. C. Romão, *Organometallics* **2009**, *28*, 2871–2879.
[27] L. Li, S. Han, Q. Li, Z. Chen, Z. Pang, *Eur. J. Inorg. Chem.* **2007**, 5127–5137.
[28] K. J. Stone, R. D. Little, *J. Org. Chem.* **1984**, *49*, 1849–1853.
[29] J. L. Petersen, J. W. Egan, *Inorg. Chem.* **1983**, *22*, 3571–3575.
[30] K. Prout, T. S. Cameron, R. A. Forder, S. R. Critchley, B. Denton, G. V. Rees, *Acta Cryst.* **1974**, *B30*, 2290–2304.
[31] M. Ishiyama, M. Shiaga, K. Sasamoto, M. Mizoguchi, P. He, *Chem. Pharm. Bull.* **1993**, *41*, 1118–1122.
[32] W. L. F. Armarego, D. D. Perrin, Purification of Laboratory Chemicals, Butterworth-Heinemann, Oxford, **1996**.
[33] J. Claffey, M. Hogan, H. Müller-Bunz, C. Pampillón, M. Tacke, *J. Organomet. Chem.* **2008**, *693*, 526–536.
[34] M. Tacke, L. P. Cuffe, W. M. Gallagher, Y. Lou, O. Mendoza, H. Müller-Bunz, F.-J. K. Rehmann, N. Sweeney, *J. Inorg. Biochem.* **2004**, *98*, 1987–1994.
[35] E. Sirignano, C. Saturnino, A. Botta, M. S. Sinicropi, A. Caruso, A. Pisano, R. Lappano, M. Maggiolini, P. Longo, *Bioorg. Med. Chem. Lett.* **2013**, *23*, 3458–3462.
[36] M. Tacke, L. T. Allen, L. Cuffe, W. M. Gallagher, Y. Lou, O. Mendoza, H. Müller-Bunz, F.-J. K. Rehmann, N. Sweeney, *J. Organomet. Chem.* **2004**, *689*, 2242–2249.
[37] L. E. Manzer, *Inorg. Chem.* **1977**, *16*, 525–528.
[38] A. Otter, H. Mühle, M. Neuenschwancler, H. P. Kellerhals, *Helv. Chim. Acta* **1979**, *62*, 1626–1631.
[39] Z. Otwinowski, W. Minor, *Methods Enzymol.* **1997**, *276*, 307–326.
[40] P. Coppens, Crystallographic Computing (Eds.: F. R. Ahmed, S. R. Hall, C. P. Huber), Munksgaard, Copenhagen, **1970**, pp. 255–270.
[41] A. Altomare, G. Cascarano, C. Giacovazzo, A. Guagliardi, M. C. Burla, G. Polidori, M. Camalli, *J. Appl. Cryst.* **1994**, *27*, 435–436.
[42] G. M. Sheldrick, SHELXL97, University of Göttingen, Germany, **1997**.
[43] H. D. Flack, *Acta Cryst.* **1983**, *A39*, 876–881.
[44] A. L. Spek, PLATON: A Multipurpose Crystallographic Tool, Utrecht University, Netherlands.