DOI: 10.1002/ejic.200600130

Characterization and Insulin-Mimetic Potential of Oxidovanadium(IV) Complexes Derived from Monoesters and -carboxylates of 2,5-Dipicolinic Acid

Jessica Gätjens,*[a][‡] Beate Meier[b] Yusuke Adachi,[c] Hiromu Sakurai,[c] and Dieter Rehder[a]

Keywords: Vanadium / N,O Ligands / Picolinates / Insulin mimesis

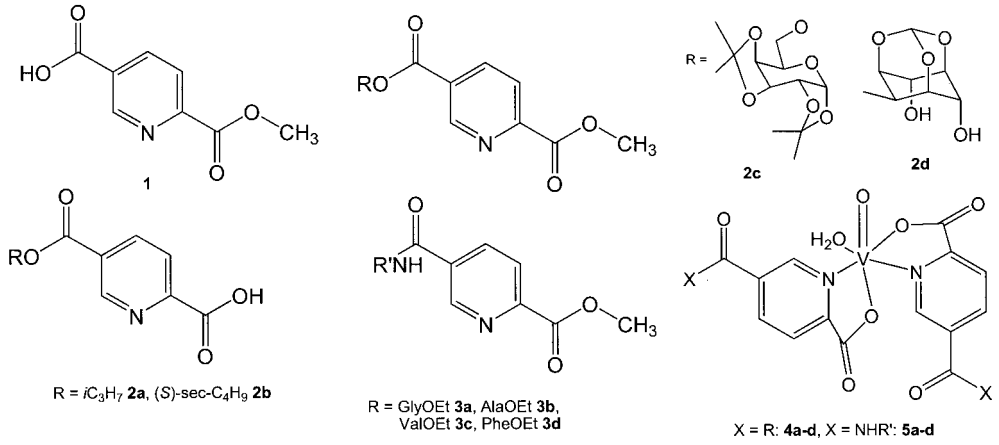
The monomethyl ester 2MeOdipicH (**1**) of 2,5-dipicolinic acid, characterized as its magnesium salt $[Mg(H_2O)_6](2MeOdipic)_2$, was converted, via the diesters 2MeO-5ROdipic and the copper complexes $[Cu(5ROdipic)_2]$, to the ligands 5ROdipicH (R = *i*Pr **2a**, (*S*)-2-Bu **2b**). The proligands 2MeO-5ROdipic (with R = diisopropyl-D-galactose **2c**, *myo*-inositol-orthoformate **2d**) and 2MeO-5R′NHdipic (where R′ represents the ethyl-protected L-amino acid residues Gly **3a**, Ala **3b**, Val **3c** and Phe **3d**) were obtained from 2-MeO-5Cldipic and the amino acid ethyl esters. Reaction of **2** and **3** with $VOSO_4$ afforded the complexes $[VO(H_2O)(5ROdipic)_2]$ (**4a–d**) and $[VO(H_2O)(5R'NHdipic)_2]$, **5a–d**, respectively. $[Mg(H_2O)_6](2MeOdipic)_2{\cdot}4H_2O$, **2b**, **3a** and **4a**$\cdot 0.5H_2O$ were characterized by single-crystal X-ray diffraction analysis. Selected type **4** and **5** complexes were submitted to in vitro tests (fibroblasts, SV 3T3 mice fibroblasts) for their uptake kinetics and insulin-mimetic behavior. The compounds were comparable to insulin in their ability to stimulate cellular glucose uptake and metabolism. In vitro tests with rat adipocytes showed that the complexes also mimic the ability of insulin to inhibit lipolysis.

Introduction

During the last two decades, in vitro and in vivo studies have demonstrated the potential of many vanadium compounds as insulin-mimetic (or insulin-enhancing) agents.[1–3] Among those which have been shown to be effective are bis(maltolato)oxidovanadium complexes,[4a] which successfully passed clinical tests phase I,[4b] $[VO(pic)_2]$ which normalizes serum glucose and fatty acid levels in rats with streptozotozin-induced diabetes type 1,[5] and $[VO_2(H_2O)(2,6\text{-dipic})]^-$, which had beneficial effects in cats suffering from diabetes type 2.[6] Picolinato complexes of vanadium appear to be generally effective,[7] the extent, however, to which they actually mimic the involvement of insulin in the glucose and lipid metabolisms is subject to variations in the coordination periphery. We have recently shown that the methyl ester derivative $[VO(H_2O)(5MeOdipic)_2]$, where 5MeOdipic is the monomethyl ester of 2,5-dipicolinate, is clearly more effective than the corresponding ethyl ester both in the ability to trigger cellular glucose uptake (and metabolism) and to inhibit lipolysis.[8] These differences in impact correlate with the net amount of vanadium taken up by the cells, possibly indicative for a balanced hydro/lipophilicity being a key factor in efficacy.

An apparent advantage of vanadium complexes in the treatment of *diabetes mellitus* type 1 (lacking insulin production) and type 2 (tolerance/resistance against insulin) is their application per os, and the possibility to design the compounds so as to provide minimal toxicity and optimal efficiency along with stability against redox- and hydrolytic break-down. The compound has to survive the acidic conditions pertinent to the stomach (pH typically around 2), as well as the slightly alkaline conditions in the small intestines (pH 7–8) and the blood stream (pH 7.35). It has to resist to a certain extent competing ligands such as the plasma constituents citrate, phosphate and transferrin in order to preserve the information implanted by the specific properties of the ligands used in the synthesis of the potential drug. The complex should be easily absorbed in the gastrointestinal tract, transported by the blood stream at least partly intact, and traffic across the cell membrane. Oxidovanadium complexes carrying monoesters of 2,5-dipicolinic acid as ligands have been shown to fulfil several of these conditions.[8] Picolinates are natural metabolites; toxicity by degradation of the complexes thus will be minimized. In order to improve the trans-membrane transport, a fine-tuning of the hydro/lipophilicity by choosing the "correct" sub-

[a] Institute of Inorganic and Applied Chemistry, University of Hamburg,
Martin-Luther-King-Platz 6, 20146 Hamburg, Germany
E-Mail: jgaetjens@yahoo.de
dieter.rehder@chemie.uni-hamburg.de
[b] bcm-research,
Entrischenbrunn 12, 85307 Entrischenbrunn, Germany
[c] Department of Analytical and Bioinorganic Chemistry, Kyoto Pharmaceutical University,
Kyoto, 607-8414, Japan
[‡] Present address: Department of Chemistry, University of Michigan,
930 N. University Ave., Ann Arbor, MI 48109, USA,
Fax: +1-734-936-7628



Scheme 1.

stituents in the ligand periphery is one option. A second option is to attach groups to the periphery for which membrane receptors exist and which can thus be recognized by the cell. Following these concepts, we have extended earlier investigations on picolinates to galactose and inositol derivatives on the one hand, and carboxamides derived from amino acids on the other hand. Scheme 1 gives an overview of the types of compounds discussed in the present work. Along with the vanadium complexes and their in vitro insulin-mimetic properties, structural features of ligands and ligand precursors will be described.

Results and Discussion

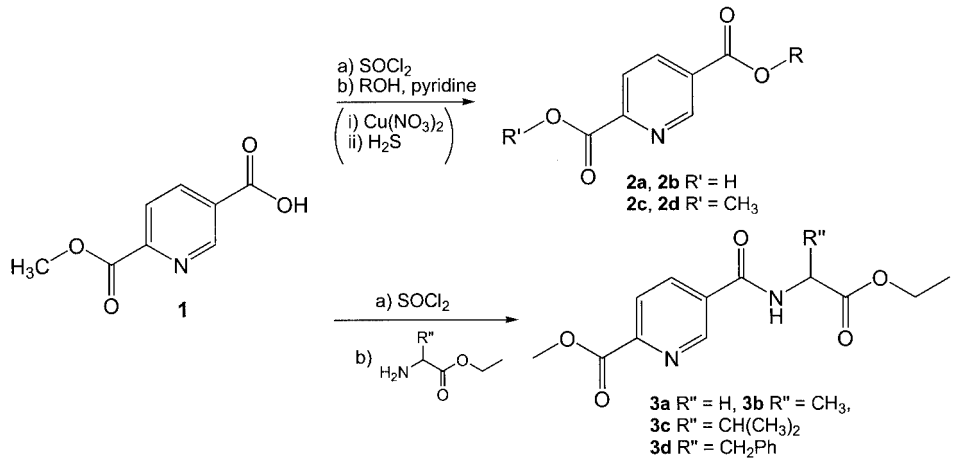
Synthesis, Characterization and Structure Determination

The syntheses of the ligands **2a**–**b** followed the route depicted in Scheme 2. 6-(Methoxycarbonyl)pyridine-3-carboxylic acid (**1**) was converted into the corresponding acyl chloride by reaction with thionyl chloride. Addition of a solution of the acid chloride in toluene to a solution of the alcohol ROH (R = *i*Pr, (*S*)-*s*Bu, in 1.4 fold excess) in pyridine yielded the mixed diesters 2MeO-5ROdipic, which were converted via the copper complexes [Cu(5ROdipic)$_2$] into the acids 5ROdipicH, **2a**–**b**. For the structure of the anion of **1**, **1**H$_{-1}$, see below.

Single crystals of **2b** were grown from aqueous acetone solution. **2b** crystallizes in the monoclinic space group $P2_1$. Bond lengths and angles are in the expected range (Table 1). The molecular structure and a picture showing the hydrogen-bonding network are displayed in Figure 1. The molecules are linked through OH···N hydrogen bonds, comparable to the features exhibited by other members of this family of dipicolinic acid derivatives.[8]

The proligands **2c**–**d** were synthesized in analogy to the ligands **2a**–**b**, with the modification that equimolar amounts of acyl chloride and alcohol were reacted. The deprotection step via the copper complexes was omitted. The cleavage of the methyl ester group occurred in situ under the conditions chosen for the synthesis of the vanadium complexes and concomitantly with the coordination of the carboxylate function to vanadium.

For the syntheses of the amino acid derivatives **3a**–**d**, cf. Scheme 2, a solution of the acyl chloride in dichloromethane was treated with a solution containing the amino acid ethyl ester hydrochloride and triethylamine in CH_2Cl_2,



Scheme 2.

Table 1. Selected bond lengths [Å] and angles [°] for $[Mg(H_2O)_6](\mathbf{1}H_{-1})_2 \cdot 4H_2O$, **2b** and **3a**.

$[Mg(H_2O)_6](\mathbf{1}H_{-1})_2 \cdot 4H_2O$		**2b**		**3a**	
C1–O1	1.4516(13)	C1–C2	1.499(4)	C1–O1	1.458(2)
C2–O1	1.3251(14)	C1–O1	1.323(4)	C2–O1	1.328(2)
C2–O2	1.2080(13)	C1–O2	1.197(4)	C2–C3	1.504(3)
C2–C3	1.4960(14)	C5–C7	1.492(4)	C6–C8	1.503(2)
C6–C8	1.5083(13)	C7–O3	1.198(4)	C8–N2	1.337(2)
C8–O3	1.2716(13)	C7–O4	1.340(4)	C8–O3	1.227(2)
C8–O4	1.2390(13)	C8–O4	1.479(4)	C9–N2	1.442(2)
C1–O1–C2	116.05(9)	O1–C1–O2	125.5(3)	O1–C2–O2	124.31(18)
O1–C2–O2	124.91(10)	O3–C7–O4	124.91(10)	O3–C8–N2	122.13(17)
O3–C8–O4	125.91(10)				

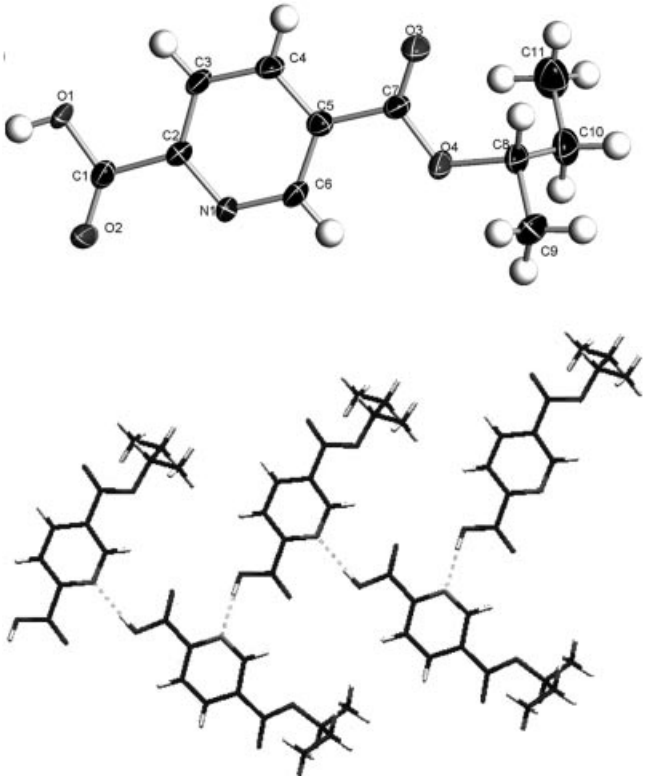


Figure 1. Top: XSHELL plot of **2b** (50% probability level). Bottom: Hydrogen bonding network for **2b** (Mercury, vers. 1.1).

which led to the formation of the desired products in good yields. The compounds were characterized by the standard spectroscopic and spectrometric methods. In the case of the glycine ethyl ester derivative **3a**, colorless single crystals were grown from dichloromethane/hexane at room temperature. **3a** crystallizes in the monoclinic space group $P2_1/c$. The molecular structure is shown in Figure 2. Bond lengths and angles are in the expected range (Table 1).

The magnesium salt of **1**, $[Mg(H_2O)_6](\mathbf{1}H_{-1})_2 \cdot 4H_2O$, was isolated in low yields as a by-product of the synthesis of the amino acid derivatives and characterized by IR and ^1H NMR spectroscopy. The magnesium apparently stemmed from $MgSO_4$ employed as a drying agent for the solvents. Single crystals grew from a methanolic solution by slow evaporation of the solvent. The compound crystallizes in the monoclinic space group $P2_1/c$ with two independent molecules in the asymmetric unit. The magnesium cation is octahedrally coordinated by six water ligands. The molecular structure is depicted in Figure 3, bonding parameters are contained in Table 2. There are hydrogen bonds between the waters of crystallization O8 and O9 (2.74 Å), O8 and the pyridine-N (2.83 Å), and O9 and water coordinated to Mg^{2+} (2.79 Å).

Following a procedure described previously,[8] the vanadium(IV) complexes **4a**–**b** were isolated from the reaction of vanadyl sulfate and the acids **2a**–**b** as green microcrystalline powders. Green single crystals of compound $\mathbf{4a} \cdot 0.5H_2O$ were obtained from a hot aqueous solution on slow cooling.

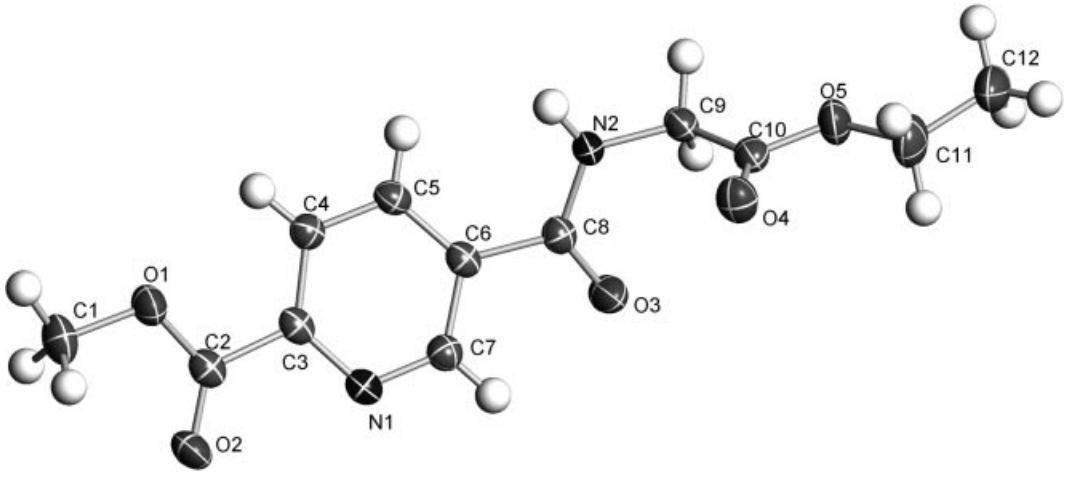


Figure 2. XSHELL plot of **3a** (50% probability level).

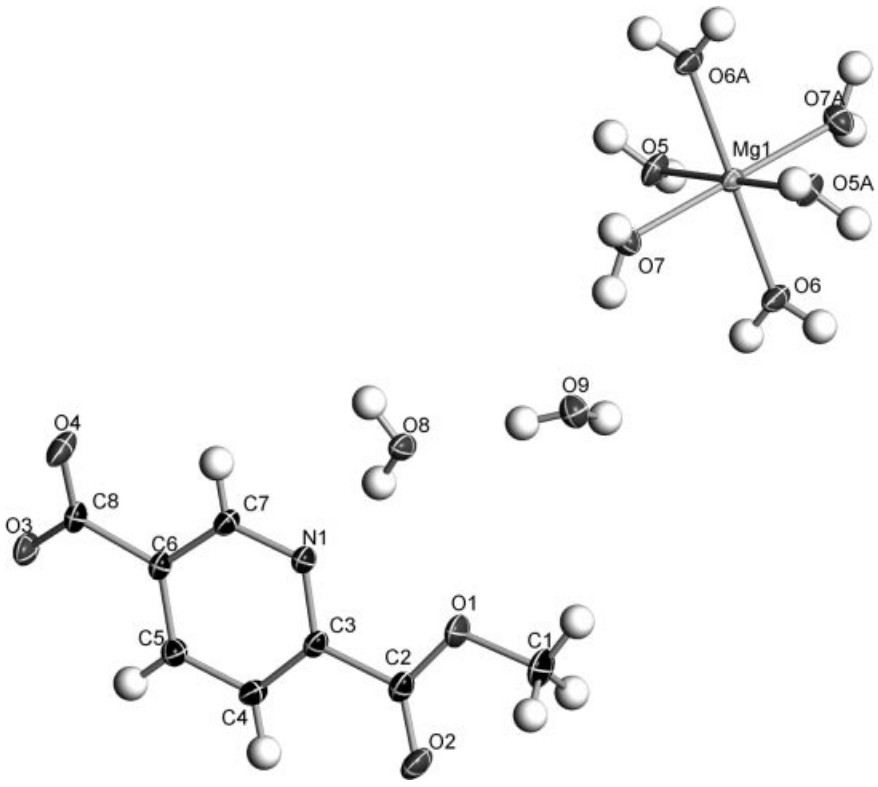


Figure 3. XSHELL plot of $[Mg(H_2O)_6](\mathbf{1}H_{-1})_2 \cdot 4H_2O$ (50% probability level). Only one of the anions $\mathbf{1}H_{-1}$ and two of the waters of crystallization are shown.

Table 2. Crystal data and structure refinement for $(\mathbf{1}H_{-1})_2$, **2b**, **3a**, **4a**.

	$[Mg(H_2O)_6](2\text{-MeOdipic})_2$	**2b**	**3a**	**4a**·0.5H_2O
Emperical formula	$C_{16}H_{32}MgN_2O_{18}$	$C_{11}H_{13}NO_4$	$C_{12}H_{14}N_2O_5$	$C_{20}H_{23}N_2O_{10.5}V$
M [g mol^{-1}]	564.75	223.23	266.25	510.35
Crystal system	monoclinic	monoclinic	monoclinic	triclinic
Space group	$P2(1)/c$	$P2(1)$	$P2(1)/c$	$P\bar{1}$
Cell dimensions				
a [Å]	13.7795(7)	4.4079(15)	13.0421(17)	10.9185(5)
b [Å]	7.5126(4)	9.434(3)	6.8948(9)	13.4119(5)
c [Å]	13.8657(8)	13.354(4)	15.465(2)	17.6792(8)
α [°]	90	90	90	69.6350(10)
β [°]	117.2500(10)	91.478(6)	112.338(2)	78.2570(10)
γ [°]	90	90	90	77.8200(10)
V [Å^3]/Z	1276.07(12)/2	555.1(3)/2	1286.3(3)/4	2348.42(17)/4
ρ(calcd.) [g cm^{-3}]	1.470	1.335	1.375	1.440
μ [mm^{-1}]	0.156	0.102	0.108	0.481
F(000)	596	236	560	996
Crystal size [mm]	0.41×0.34×0.10	0.55×0.26×0.07	0.65×0.10×0.07	0.50×0.250×0.10
θ range [°]	2.94–32.50	2.64–27.49	1.69–27.49	2.14–29.00
Index ranges	–20<h<20, –11<k<11, –20<l<20	–5<h<5, –12<k<12, –16<l<16	–16<h<16, –8<k<8, –19<l<19	–14<h<14, –18<k<18, –24<l<24
Reflections collected	33675	6338	14783	57316
Independent reflections (R_{int})	4587 (0.0457)	1328 (0.0746)	2916 (0.0460)	12361 (0.0478)
Restraints/parameters	15/200	1/148	0/175	13/661
GooF on F^2	1.004	0.928	1.055	0.969
Final R ($I > 2\sigma I_0$), R_1/wR_2	0.0414/0.0985	0.0473/0.0871	0.0464/0.0942	0.0470/0.1208
R indices (all data), R_1/wR_2	0.0582/0.1050	0.0694/0.0932	0.0765/0.1200	0.0748/0.1348
Largest diff. peak and hole [e·Å^{-3}]	0.555/–0.259	0.225/–0.184	0.518/–0.430	1.099/–0.521

The compound crystallizes in the monoclinic space group $P\bar{1}$ with two independent molecules and one water of

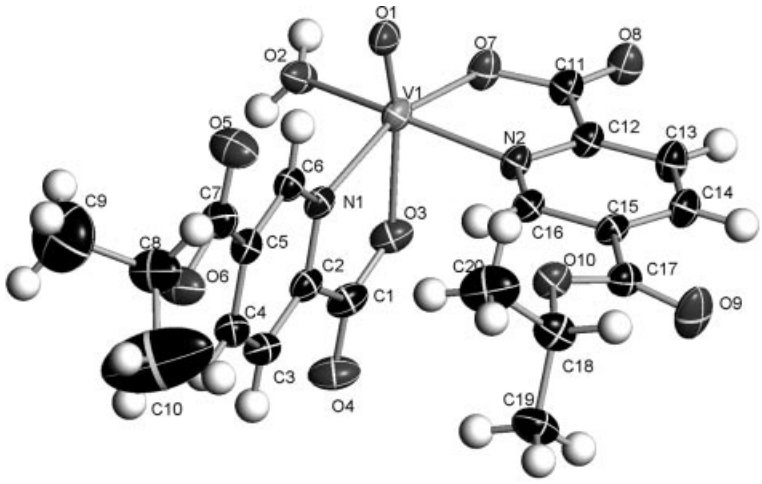


Figure 4. XSHELL plot of **4a** (50% probability level). Only one of the two independent molecules, containing one of the *i*Pr substituents in a disordered state, is shown. Water of crystallization omitted. Selected bonding parameters: V1–O1 1.5989(14), V1–O2 2.0175(14), V1–O3 1.1290(15), V1–O7 1.9890(15), V1–N1 2.1503(17), V1–N2 2.1072(16) Å; O1–V1–O3 164.55(7), O2–V1–N2 164.62(7), O7–V1–N1 161.62(6)°.

crystallization, disordered over two positions, in the asymmetric unit. Two ligands coordinate in the expected bidentate manner to form, together with an aqua ligand (O2) in the equatorial plane and an oxo ligand (O1) in the apical position, a distorted octahedron, Figure 4. The oxygen O3 of one of the carboxylato groups occupies the axial position *trans* to the oxo ligand, leading to the rather long V1–O3 distance 2.1290(15) Å due to the *trans* influence of the doubly bonded oxygen. The isopropyl group of one of the ligands is disordered over two positions (1:1) in one of the two independent molecules. The bonding parameters for the coordinated ligand anion are the same within the error limits as in the free ligand **2a** (Table 1). The water of crystallization is in hydrogen-bonding contact with the carboxylate oxygens O4 (2.79 Å) and O3 (2.83 Å).

For the synthesis of the vanadium(IV) complexes **4c**–**d** and **5a**–**d**, an aqueous solution of vanadylsulfate was mixed with a solution of the proligand and sodium acetate in THF/water and heated to reflux for 6 h. Under these conditions, the deprotection of the 2-position of the ligand precursor and the subsequent formation of the desired vanadium complex took place. The resulting dark green solution was evaporated to dryness. Recrystallization from THF yielded complexes **4c**–**d** and **5a**–**d** as green solids. All analyses confirmed the coordination of two ligand molecules to the metal center plus a solvent molecule, commonly water; cf. Scheme 1 and **4a** in Figure 4.

Insulin-Mimetic Tests

The biological tests with regard to glucose uptake and metabolism were carried out with a modified (see below) MTT reduction assay using Simian virus transformed mice fibroblasts as described earlier.[16] In this assay, yellow soluble MTT is reduced to insoluble formazan blue by reduction equivalents generated by the metabolism of glucose by the mitochondrial respiratory chain. The amount of formazan blue is determined photometrically and correlates with the ability of either insulin or the vanadium compound to stimulate cellular glucose uptake and metabolism by the cells. The cells were grown to sub-confluency, and incubated in insulin-free medium for 24 h in order to minimize effects other than those imparted by the vanadium compound. This procedure was followed by 3 h incubation with the medium supplemented with the vanadium compound. This modified MTT test has previously been verified using D-[C6–^{3}H]glucose.[16] In order to determine the time dependent effect, a sample was taken every 15–30 minutes. Compounds included in this study are **4c**, **5a**, **5c**, **5d**, $VOSO_4$, and [VO(5MeOdipic)$_2$], the insulin-mimetic activity of which has been established earlier, and which proved most effective among the alkyl derivatives [VO(5ROdipic)$_2$].[8] The amino acid derivative with alanine, **5b**, displayed a behavior similar to the glycine derivative **5a**.

Figure 5 shows the absorbance for different concentrations of the vanadium compounds relative to a control group (without vanadium or insulin), and cells incubated with insulin instead of the vanadium compound. The data after 60 minutes incubation time are shown. During this time, about 90% of the overall effect is observed (see below). Except for the glycine derivative **5a**, all compounds exhibit insulin-mimetic properties in the concentration range (1 μM $\leq c \leq$ 200 μM) tested. The most promising results are those for the phenylalanine derivative **5d**, showing comparable stimulation of glucose uptake and metabolism as insulin. Particularly noteworthy is the fact that even at rather low concentrations ($c <$ 10 μM) the vanadium complexes are still active (Figure 5).

The time-dependent effect of compounds **4c** and **5a**, [VO(5MeOdipic)$_2$] and [VO(pic)$_2$] is shown in Figure 6. At an intermediate vanadium concentration of c = 40 μM the maximum level of activity is reached after an incubation time of approximately 90 minutes. Similar results have been found for other concentrations and other complexes. The

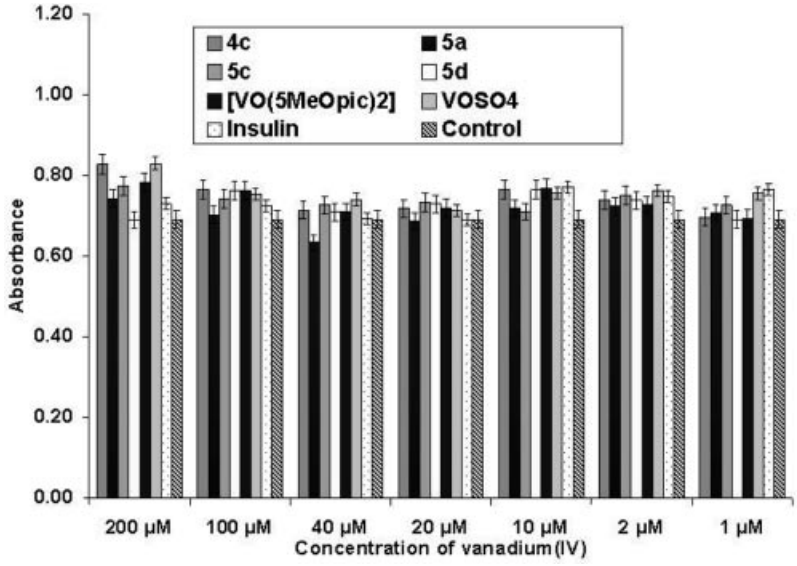


Figure 5. Glucose uptake stimulated by the vanadium picolinates **4c**, **5a**, **5c** and **5d** after 60 min. The absorbance, shown relative to a control group, reflects the efficacy of the compounds. The diagram also shows the data for $[VO(5MeOdipic)_2]$, $VOSO_4$, and a group of cells where insulin was added instead of vanadium.

time dependence in activity for the compounds apparently is not as significant as one might expect when considering the differences in the ligand spheres. Further, Figure 6 clearly shows that the galactose derivative **4c** exhibits, at this specific concentration, an insulin-mimetic activity comparable to the well established efficiency of $[VO(pic)_2]$[5] and $[VO(5MeOdipic)_2]$.[8] The time dependent insulin-mimetic effect of **4c** at different concentrations reveals that the maximum level of activity at high concentrations (c = 400 µM and 200 µM) is already reached after an incubation time of approx. 45 minutes, cf. Figure 7. In this respect, **4c** is unique, since all of the other compounds considered in this study displayed the highest activity after 60 to 90 minutes, even at high concentrations (not shown). Furthermore, the effect promoted by **4c** at c = 400 µM and 200 µM is significantly higher than the activity of insulin itself.

Testing of compounds **4c** and **5a**–**c** with respect to their ability to inhibit lipolysis in rat adipocytes required the presence of 1 mM ascorbic acid as a reducing agent. In the absence of ascorbic acid, aerial oxidation (^{51}V NMR evidence) in the Krebs–Ringer buffer to species of low activity occurred. Figure 8 shows the effects on the release of free fatty acids (FFA) by $[V^{IV}O(pic)_2]$, the four vanadium(IV) complexes **4c** and **5a**–**c**, and the two dioxidovanadium(V) complexes $K[VO_2(pic)_2]$ and $K[VO_2(2,5dipic)_2]$. All of the compounds show an inhibitory effect with respect to the control C (epinephrine [= adrenalin], which works as an effective antagonist to insulin), an effect which increases as concentration is increased from 0.1 to 1 mM. The results obtained with insulin (B in Figure 8) are not quite reached. There is no sizeable difference in efficiency between the neutral oxidovanadium(IV) and the anionic dioxidovanadi-

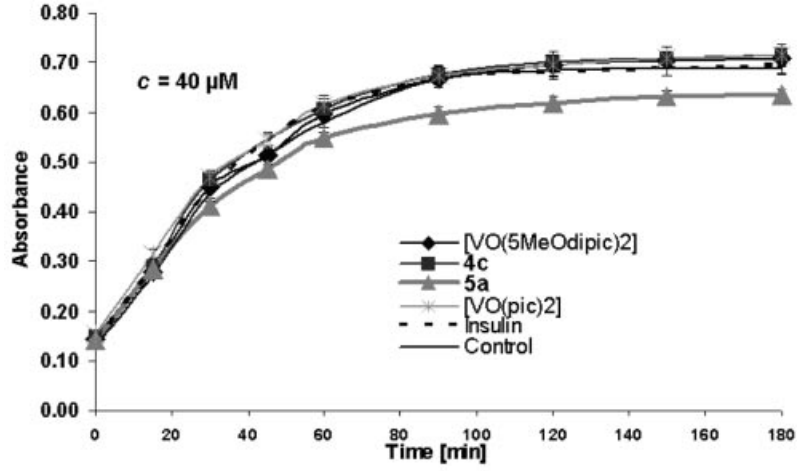


Figure 6. Glucose uptake stimulated by **4c** and **5a** (c = 40 µM) presented as a function of time. This diagram also includes the results for $[VO(pic)_2]$, $[VO(5MeOdipic)_2]$ and insulin. Data are not corrected for background.

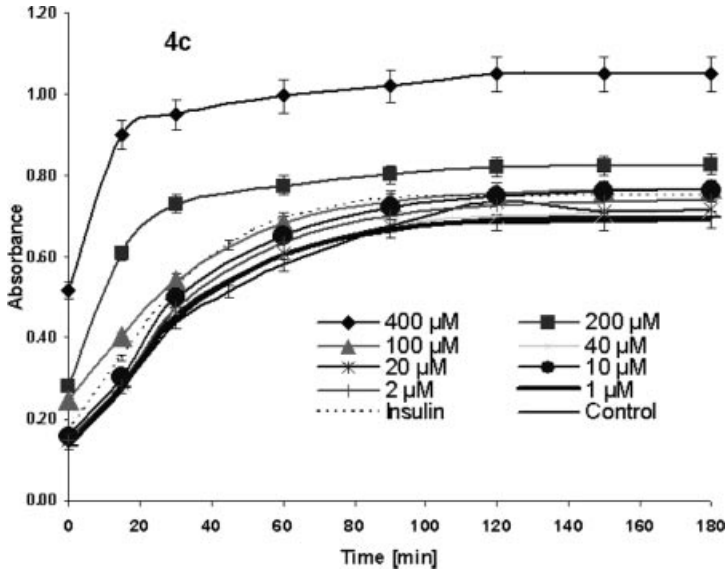


Figure 7. Glucose uptake stimulated by the galactosyl derivative **4c** as a function of time over a wide concentration range. Data are not corrected for background.

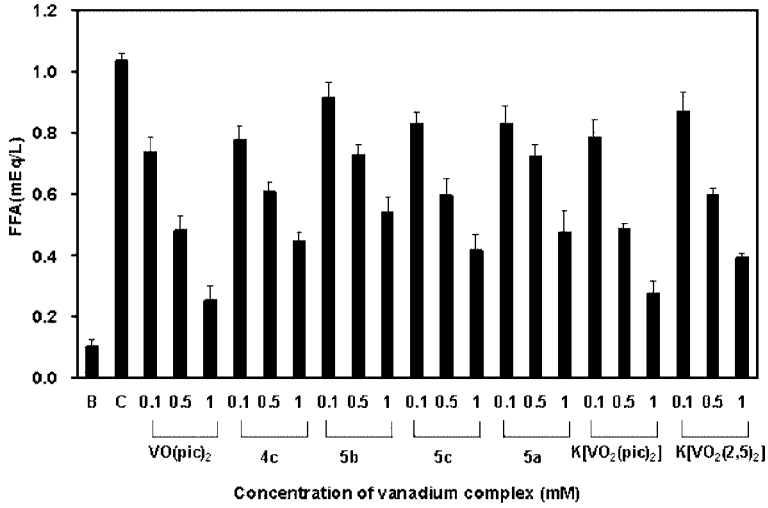


Figure 8. Inhibitory effects of vanadium complexes on FFA-release from rat adipocytes treated with epinephrine. Data are expressed as the means ± SDs for three experiments. B: adipocytes treated with insulin. C: adipocytes pre-incubated with saline for 30 min before treatment with 10 µM epinephrine for 3 h.

um(V) compounds, nor between the different picolinate derivatives. The ligands themselves did not show any appreciable activity (data not shown).

Conclusions

In extension of earlier work,[8] we have introduced here a new concept for the design of potentially insulin-mimetic vanadium(IV) compounds. The framework represented by pyridine-2,5-dicarboxylic acid was extended by introducing, into the 5-position, organic molecules into the ligand sphere for which membrane receptors exist and/or which modify the hydro/lipophilicity of the dipicolinatovanadium complexes. Here, we have employed amino acids, galactose and inositol residues, along with alkyl derivatives for comparison. Selected compounds were subjected to in vitro studies of their ability to stimulate glucose uptake and oxidative degradation by modified fibroblasts on the one hand, and to inhibit lipolysis by adipocytes on the other hand. Except for the glycine and L-alanine derivatives, all compounds were effective insulin-mimetics in the glucose uptake experiments in the concentration range 200 to 1 µM. The galactose containing complex initiated an earlier saturation of glucose uptake in the time-dependent studies than other compounds, which can be interpreted in terms of a faster uptake of the galactosyl complex by the cells. All of the complexes also showed insulin-mimetic activity in the inhibition of li-

polysis, in particular at relatively high concentrations of 1 mM.

Experimental Section

General and Chemicals: All preparations of vanadium complexes in the oxidation state +IV were carried out under nitrogen atmosphere, using Schlenk techniques. For further handling of the complexes, Schlenk techniques were dispensable. Chemicals were obtained from Merck, Aldrich and Fluka and used without further purification unless stated otherwise. Solvents were dried and purified by standard procedures. Tetrahydrofuran (THF) was dried and deoxygenated by refluxing for at least three hours over $LiAlH_4$ and distilled in an N_2 stream. Triethylamine was distilled (88–90 °C) and stored over molecular sieves (4 Å). Dichloromethane was refluxed for 24 h over calcium hydride. Toluene was refluxed for 24 h over sodium and then distilled in an N_2 stream. Pyridine ($H_2O \leq 0.005\%$) was stored over molecular sieve. Deionized water was degassed before use. Products were dried at room temperature under vacuum and stored under N_2. The following compounds were prepared according to published procedures: 6-(methoxycarbonyl)-pyridine-3-carboxylic acid (**1**),[9] methyl 5-(chlorocarbonyl)pyridine-2-carboxylate,[10] *myo*-inositol-orthoformate,[11,12] 1,2:3,4-di-*O*-isopropylidene-*α*-D-galactose,[13] [VO(pic)$_2$],[14]K[VO_2(pic)$_2$],[14] K[VO_2(2,5dipic)].[15]

Analyses and Methods: Elemental analyses were carried out in the Analytical Laboratory of the Chemistry Department, University of Hamburg. Infrared spectra were recorded as KBr pellets on a Perkin–Elmer 1720 FT–IR spectrometer. NMR spectra were obtained either on a Varian Gemini 200 BB or a Bruker Avance 400 spectrometer at room temperature in 5 mm tubes. Chemical shifts δ are reported in ppm (parts per million) relative to TMS for ^1H and ^{13}C NMR spectra. Coupling constants (J) are quoted in Hertz. Mass spectrometry was carried out at the Institute of Organic Chemistry, University of Hamburg, with the usual spectrometer settings on a Varian MAT 311A (70 eV, EI) or a VG Analytical 70–250 S (FAB). EPR spectra of **4a**–**d** and **5a**–**d** were scanned at ca. 9.6 GHz (X-band) on a Bruker ESP-300 E spectrometer at room temperature and 100 K. Parameter adaptations by simulation were achieved by using the Bruker software SimFonia. X-ray structure analyses were carried out at 153(2) K using Mo-K_α irradiation (λ = 0.71073 Å, graphite monochromator) on a Smart Apex CCD diffractometer. Hydrogen atoms were usually found, and otherwise calculated into idealized positions and included in the last cycles of refinement. Absorption corrections were carried out by SADABS. Structure solution and refinement were carried out using the SHELXTL-Plus software package (G. M. Sheldrick, Version 5.1, Bruker AXS, 1998). For crystal data and structure refinement see Table 2.

CCDC-22102, -285853, -225187, and -257605 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.

Insulin-Mimetic Tests: Tests for the glucose uptake activity were based on the MTT (MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) reduction assay.[16] The cells were cultured in glucose-rich DMEM medium (Dulbecco's modification of Eagle's medium) supplemented with 10% fetal calf serum at 37 °C/5% CO_2 to subconfluency. Afterwards, the cells were passed to an insulin-free, DMEM high glucose medium for 24 h. For the MTT tests, DMEM (without phenol red) supplemented with MTT (Sigma; 0.5 g/L) and solutions of the vanadium complexes in different concentrations were added to the cells and incubated for up to 3 h at 37 °C in a 5% CO_2 atmosphere. The cells were separated from the medium and the reaction was stopped by addition of 0.1 M HCl in 2-propanol. The insoluble dye was extracted by HCl/2-propanol, and the amount of formazan blue formed by reduction of MTT was measured at 570 nm in a multi-well reader (SLT 340 ATC). All measurements were carried out in triplicate; standard deviations for the absorbances typically amount to 4 ± 2%.

Tests for the inhibition of free fatty acid (FFA) release were performed on epidymal fat pads, excised from male Wistar rats (7 weeks) anesthetized with diethyl ether. The pads were cut into appropriately sized pieces and were incubated with collagenase in KRB buffer (Krebs–Ringer hydrogen carbonate buffer; 120 mM NaCl, 1.27 mM $CaCl_2$, 1.2 mM $MgSO_4$, 4.75 mM KCl, 1.2 mM KH_2PO_4, and 24 mM $NaHCO_3$; pH 7.4) containing 2% BSA at 37 °C with gentle shaking at 100 cycle/min for 1 h. At the end of the incubation period, the prepared cells were filtered through sterilized cotton gauze and washed three times with the KRB buffer. The cells were incubated at 37 °C for 30 min with the vanadium complexes, dissolved in DMSO, at various concentrations (0.1–1.0 mM), including 1 mM ascorbic acid to prevent their oxidations in KRB buffer containing 2% DMSO. A 10 μM solution of epinephrine was then added to the reaction mixtures, and the resulting solutions were incubated at 37 °C for 3 h. The mixtures were centrifuged at 3000 rpm for 10 min at 4 °C. On the outer solution of the cells, the FFA level was determined with a FFA kit (NEFA C test; Wako, Osaka, Japan).

All animal experiments in the present study were approved by the Experimental Animal Research Committee of Kyoto Pharmaceutical University (KPU), and were performed according to the Guideline for Animal Experimentation of the KPU.

Synthesis and Characterization

For the NMR assignments, the numbering of the ring-H and -C atoms follows the usual one. C1 and C7 refer to the carboxylic carbons in the ring positions 2 and 5, respectively.

[$Mg(H_2O)_6$](1H$_{-1}$)$_2$: This compound was obtained in low yields as a by-product during the syntheses of the amino acid derivatives **3a**–**d** (see below). Single crystals were grown by slow evaporation of a methanolic solution. IR (KBr) $\tilde{\nu}$ = 3114, 3075 (ar. C–H); 2955, 2924, 2851 (C–H); 1730 (sh), 1708 (C=O); 1636, 1605, 1561 (C=C), (C=N); 1479, 1439, 1391, 1324 (C–H); 1287, 1258 (C=C), (C=N); 1158, 1122, 1072, 1030, 1005 (C–O–C); 818 (ar. C–H), ν(C–CO_2); 746 (ar. C–H) cm^{-1}. ^1H NMR (400 MHz, CD_3OD/TMS): δ = 9.19 (m, 1 H, H-2), 8.47–8.45 (m, 1 H, H-4), 8.18–8.16 (m, 1 H, H-5), 3.98 (s, 3 H, H-1) ppm.

General Procedure for the Syntheses of the Ligands 2a–b: To a solution of methyl 5-(chlorocarbonyl)pyridine-2-carboxylate (1.5 g, 7.56 mmol) in toluene (5 mL) was added dropwise and with cooling a 1.4 fold excess of the alcohol in pyridine (15 mL). After the addition of a catalytic amount of 4-(dimethylamino)pyridine, the resulting dark solution was stirred overnight at room temperature. The solvent was evaporated and the residue dissolved in water. The aqueous solution was extracted with dichloromethane (5 × 30 mL). The organic layer was dried with Na_2SO_4 and the solvent evaporated. Purification was carried out using column chromatography on silica gel, elutant ethyl acetate/*n*-hexane, 1:1, to give a light yellow solid (yield 59–65%) which was treated with an equimolar amount of $Cu(NO_3)_2 \cdot 3H_2O$, followed by treatment with hydrogen sulfide as has been described earlier[8] to produce the ligands as white solids in yields of 50–85%.

5-(Isopropyloxycarbonyl)-2-pyridinecarboxylic Acid (2a): Yield 44% (0.69 g). $C_{10}H_{11}NO_4$ (209.20): calcd. C 57.41, H 5.30, N 6.70; found

C 57.34, H 5.31, N 6.67. IR (KBr) $\tilde{\nu}$ = 3053 (ar. C–H); 2980, 2875, 2767 (C–H); 1718 (C=O); 1600, 1583, 1498 (C=C), (C=N); 1471, 1459, 1377 (C–H), (O–H); 1301, 1269 (C=C), (C=N); 1108, 1036 (C–O–C); 750 (ar. C–H) cm^{-1}. ^1H NMR (200 MHz, [D$_6$]DMSO): δ = 9.12 (dd, $^4J_{6,4}$ = 2.17 Hz, $^5J_{6,3}$ = 0.82 Hz, 1 H, H-6), 8.40 (dd, $^4J_{4,6}$ = 2.17 Hz, $^3J_{4,3}$ = 8.10 Hz, 1 H, H-4), 8.13 (dd, $^5J_{3,6}$ = 0.82 Hz, $^3J_{3,4}$ = 8.10 Hz, 1 H, H-3), 5.16 (h, ^3J = 6.23 Hz, 1 H, C*H*(CH$_3$)$_2$), 1.32 (d, ^3J = 6.23 Hz, 6 H, CH(C*H*$_3$)$_2$) ppm. ^{13}C NMR (50 MHz, [D$_6$]DMSO): δ = 166.17 (C-1), 164.21 (C-7), 152.32 (C-2), 150.39 (C-6), 138.82 (C-4), 128.98 (C-5), 125.24 (C-3), 69.95 (*C*H(CH$_3$)$_2$), 22.21 (CH(*C*H$_3$)$_2$) ppm. MS (70 eV, EI): *m*/*z* (%): 209 (5) ($M^{+\cdot}$), 168 (42) (M – C_3H_5), 165 (34) (M – CO_2), 150 (100) (M – C_3H_7O), 123 (41) (M – $C_4H_6O_2$), 43 (21) ($C_2H_4N^+$).

5-[(*S*)-*sec*-Butyloxycarbonyl]-2-pyridinecarboxylic Acid (2b): Yield 50% (0.84 g). $C_{10}H_{11}NO_4$ (223.23): calcd. C 59.19, H 5.87, N 6.27; found C 58.88, H 5.91, N 6.31. IR (KBr) $\tilde{\nu}$ = 3047 (ar. C–H); 2976, 2935, 2878, 2768 (C–H); 1715 (C=O); 1599, 1581 (C=C), (C=N); 1457, 1419, 1372 (C–H), (O–H); 1297, 1267 (C=C), (C=N); 1119, 1109, 1035 (C–O–C); 749 (ar. C–H) cm^{-1}. ^1H NMR (200 MHz, [D$_6$]DMSO): δ = 9.17 (br. m, 1 H, H-6), 8.46 (m, 1 H, H-4), 8.19 (br. m, 1 H, H-3), 5.06 (tq, ^3J = 6.2 Hz, 1 H, C*H*(CH$_3$)CH$_2$CH$_3$), 1.78–1.64 (m, 2 H, CH(CH$_3$)C*H*$_2$CH$_3$), 1.33 (d, ^3J = 6.23 Hz, 3 H, CH(C*H*$_3$)CH$_2$CH$_3$), 0.94 (t, ^3J = 7.4 Hz, 3 H, CH(CH$_3$)-CH$_2$C*H*$_3$) ppm. ^{13}C NMR (50 MHz, [D$_6$]DMSO): δ = 165.87 (C-1), 163.84 (C-7), 151.9 (C-2), 148.88 (C-6), 138.24 (C-4), 128.26 (C-5), 124.75 (C-3), 73.63 (*C*H(CH$_3$)CH$_2$CH$_3$), 28.26 (CH(CH$_3$)-*C*H$_2$CH$_3$), 19.21 (CH(*C*H$_3$)CH$_2$CH$_3$), 9.53 (CH(CH$_3$)-CH$_2$*C*H$_3$) ppm. MS (70 eV, EI): *m*/*z* (%): 223 (1) ($M^{+\cdot}$), 168 (60) (M – C_4H_7), 150 (100) (M – C_4H_9O), 122 (25) (M – $C_5H_9O_2$).

Preparation of the Galactose/Inositol Derivatives 2c, 2d: To a solution of methyl 5-(chlorocarbonyl)pyridine-2-carboxylate (600 mg, 3.0 mmol) in toluene (10 mL) in an ice bath was added a solution of the corresponding alcohol (3.0 mmol) in pyridine (15 mL). After addition of a catalytic amount of 4-(dimethylamino)pyridine, the resulting solution was stirred for 12 h at room temperature. The solvent was evaporated and the residue extracted with toluene (10 mL), followed by dichloromethane (10 mL). The galactose derivative was dissolved in 1-butanol and the organic phase washed with water and a diluted solution of Na_2CO_3. The organic layer was dried with $MgSO_4$ and the solvent was evaporated to yield a yellow oil. The inositol derivative was dissolved in water and the aqueous phase was extracted with ethyl acetate. The organic layer was dried with $MgSO_4$ and the solvent was evaporated to yield a light yellow solid.

2MeO-5GalOdipic (2c): Yield 89% (0.98 g). $C_{20}H_{25}NO_9{\cdot}2H_2O{\cdot}0.5(C_4H_{10}O)$ (496.51): calcd. C 53.22, H 6.90, N 2.82; found C 53.56, H 6.52, N 2.66. IR (KBr) $\tilde{\nu}$ = 3112, 2989 (ar. C–H); 2933, 2851 (C–H); 1728, 1716 (sh) (C=O); 1627, 1597, 1580, 1479 (C=C), (C=N); 1439, 1384 (C–H); 1311, 1275, 1255, 1213, 1168, 1139, 1071, 1003 (C–O), (C–N), (C–O–C); 748 (ar. C–H) cm^{-1}. ^1H NMR (400 MHz, CD$_3$OD/TMS): δ = 9.22–9.15 (m, 1 H, H-7), 8.56–8.53 (m, 1 H, H-5), 8.27–8.22 (m, 1 H, H-4), 5.52–5.50 (m, 1 H, H-1′), 4.64–4.61 (m, 1 H, H-5′), 4.36–4.28 (m, 2 H, H-2′, H-3′), 4.02 (s, 3 H, H-1), 3.89–3.85 (m, 1 H, H-4′), 3.69–3.66 (m, 2 H, H-6′), 1.53, 1.41, 1.34, 1.33 (s, 12 H, H-9′, H-10′, H-11′, H-12′) ppm. ^{13}C NMR (100 MHz, CD$_3$OD/TMS): δ = 165.9, 165.4 (C-8, C-2), 151.8 (C-3), 151.3 (C-7), 140.0 (C-5), 130.3 (C-6), 126.1 (C-4), 110.8, 109.7 (C-7′, C-8′), 97.7 (C-1′), 72.4 (C-2′), 72.2 (C-3′), 71.9 (C-4′), 67.5 (C-5′), 66.0 (C-6′), 53.5 (C-1), 26.3, 26.2, 25.1, 24.6 (C-9′, C-10′, C-11′, C-12′) ppm. MS (70 eV, EI): *m*/*z* (%): 408 (100) (M – CH_3), 183 (27) ($C_8H_9NO_4^+$), 164 (50) ($C_8H_6NO_3^+$), 115 (26) ($C_6H_{11}O_2^+$), 100 (45) ($C_5H_8O_2^+$), 85 (24) ($C_5H_9O^+$), 81 (57) ($C_5H_5O^+$), 59 (29) ($C_2H_3O_2^+$), 43 (53) ($C_3H_7^+$).

2MeO-5-Inodipic (2d): Yield 69% (0.73 g). $C_{15}H_{15}N_{1.3}O_9$ (357.48): calcd. C 50.39, H 4.23, N 5.09; found C 51.90, H 4.35, N 5.18. IR (KBr) $\tilde{\nu}$ = 3399, 3326 (O–H), 3013 (ar. C–H); 2952, 2924, 2852 (C–H); 1729, 1717 (C=O); 1627, 1597, 1580, 1478 (C=C), (C=N); 1440, 1385 (C–H); 1275, 1162, 1139, 1060, 1007, 993, 960 (C–O), (C–N), (C–O–C); 748 (ar. C–H) cm^{-1}. ^1H NMR (200 MHz, CD$_3$OD/TMS): δ = 9.21–9.20 (m, 1 H, H-7), 8.28–8.22 (m, 1 H, H-5), 7.98–7.88 (m, 1 H, H-4), 5.41 (d, ^4J = 1.28 Hz, 1 H, H-7′), 4.44–4.40 (m, 2 H, H-1′, H-4′), 4.18–4.06 (m, 4 H, H-2′, H-3′, H-5′, H-6′), 4.01 (s, 3 H, H-1) ppm. ^{13}C NMR (100 MHz, CD$_3$OD/TMS): δ = 166.9, 165.9 (C-8, C-2), 151.5 (C-7), 147.9 (C-3), 140.3 (C-5), 131.3 (C-6), 126.1 (C-4), 103.9 (C-7′), 76.0 (C-2′, C-6′), 70.6 (C-4′), 69.1 (C-3′, C-5′), 61.1 (C-1′), 53.5 (C-1) ppm. MS (70 eV, EI): *m*/*z* (%): 352 (3) ($M^{+\cdot}$-H), 236 (10) (M – C_7H_3NO), 182 (44) ($C_8H_8NO_4^+$) 164 (100) ($C_8H_6NO_3^+$), 137 (26) ($C_7H_7NO_2^+$), 106 (20) ($C_6H_4NO^+$), 73 (41) ($C_3H_5O_2^+$), 44 (41) (CO_2^+).

General Procedure for Compounds (2MeO-5R′NHdipic) 3a–d: To a solution of the L-amino acid ethyl ester hydrochloride (5.0 mmol) in dichloromethane (10 mL) was added triethylamine (1.52 mL, 1.11 g, 11.0 mmol). The resulting mixture was stirred for 5 minutes (sometimes formation of a white precipitate was observed). Then a solution of methyl 5-(chlorocarbonyl)pyridine-2-carboxylate (1.0 g, 5.0 mmol) in dichloromethane (10 mL) was added drop wise with cooling (5–10 °C). The resulting yellow solution was stirred for 12 h at room temperature. The solvent was removed by evaporation and the residue was dissolved in ethylacetate. The solution was filtered, and the precipitate washed with a small amount of ethylacetate. The filtrate was concentrated. Purification was carried out using column chromatography on silica gel; elutant ethyl acetate/*n*-hexane, 1:1. The products were isolated as slightly yellow oils, which solidified upon cooling. Yields were 36 to 66%. Single crystals of compound **3a** were obtained by slow diffusion of *n*-hexane into a saturated solution of the compound in dichloromethane.

2MeO-5GlyNHdipic (3a): Yield 36% (0.50 g). $C_{12}H_{14}N_2O_5$ (266.25): calcd. C 54.13, H 5.30, N 10.52; found C 54.15, H 5.57, N 9.71. IR (KBr): $\tilde{\nu}$ = 3355 (N–H); 3063 (ar. C–H); 2977, 2961 (C–H); 1753, 1727, 1664 (C=O); 1595, 1531, 1480 (C=C), (C=N), (N–H); 1440, 1383 (C–H); 1311, 1253, 1200, 1169, 1128, 1024 (C–O), (C–N), (C–O–C); 753 (ar. C–H) cm^{-1}. ^1H NMR (400 MHz, CDCl$_3$/TMS): δ = 9.14–9.13 (m, 1 H, H-7), 8.33–8.30 (m, 1 H, H-5), 8.21–8.19 (m, 1 H, H-4), 7.36 (br. t, 1 H, N*H*), 4.28–4.23 (m, 4 H, H-9, H-11), 4.03 (s, 3 H, H-1), 1.31 (t, $^3J_{12,11}$ = 7.1 Hz, 3 H, H-12) ppm. ^{13}C NMR (100 MHz, CDCl$_3$/TMS): δ = 169.74 (C-8), 164.96 (C-2), 164.90 (C-10), 149.81 (C-3), 148.36 (C-7), 136.63 (C-5), 132.22 (C-6), 124.77 (C-4), 61.78 (C-11), 53.18 (C-1), 41.90 (C-9), 14.13 (C-12) ppm. MS (70 eV, EI): *m*/*z* (%): 266 (6) ($M^{+\cdot}$), 208 (33) (M – C_3H_6O), 193 (13) (M – $C_3H_5O_2$), 164 (100) (M – $C_5H_{10}O_2$), 40 (16) ($C_3H_4^+$).

2MeO-5AlaNHdipic (3b): Yield 66% (0.92 g). $C_{13}H_{16}N_2O_5$ (280.28): calcd. C 55.71, H 5.75, N 9.99; found C 55.74, H 5.52, N 9.74. IR (KBr) $\tilde{\nu}$ = 3296 (N–H); 3067 (ar. C–H); 2985, 2951, 2854 (C–H); 1744, 1718, 1645 (C=O); 1595, 1540 (C=C), (C=N), (N–H); 1455, 1442, 1381 (C–H); 1311, 1291, 1246, 1214, 1177, 1136, 1020 (C–O), (C–N), (C–O–C); 744 (ar. C–H) cm^{-1}. ^1H NMR (400 MHz, CDCl$_3$/TMS): δ = 9.13–9.12 (m, 1 H, H-7), 8.28–8.21 (m, 2 H, H-4, H-5), 6.94 (br. d, 1 H, N*H*), 4.82–4.75 (dq, $^3J_{9,NH}$ = 7.1 Hz, $^3J_{9,10}$ = 7.14 Hz, 1 H, H-9), 4.27 (q, $^3J_{12,13}$ = 7.14 Hz, 2 H, H-12), 4.04 (s, 3 H, H-1), 1.56 (d, $^3J_{10,9}$ = 7.14 Hz, 3 H, H-10), 1.33 (t, $^3J_{13,12}$ = 7.14 Hz, 3 H, H-13) ppm. ^{13}C NMR (50 MHz, CDCl$_3$/TMS): δ = 172.90 (C-8), 164.86 (C-2), 164.25 (C-11), 149.74 (C-3), 148.41 (C-7), 136.43 (C-5), 132.27 (C-6), 124.81 (C-4), 61.82 (C-12), 53.16 (C-1), 48.83 (C-9), 18.09 (C-10), 14.12 (C-13) ppm.

MS (70 eV, EI): *m/z* (%): 280 (33) ($M^{+\cdot}$), 235 (13) (M – C_2H_5O), 208 (34) (M – $C_3H_4O_2$), 207 (100) (M – $C_3H_5O_2$), 165 (33) (M – $C_6H_{11}O_2$), 164 (76) (M – $C_6H_{12}O_2$), 136 (21) (M – $C_6H_{10}NO_3$), 106 (20) (M – $C_7H_{12}NO_4$), 78 (25) ($C_5H_4N^+$).

2MeO-5ValNHdipic (3c): Yield 66% (1.01 g). $C_{15}H_{20}N_2O_5$ (308.33): calcd. C 58.43, H 6.54, N 9.09; found C 58.43, H 6.25, N 8.94. IR (KBr) $\tilde{\nu}$ = 3307 (N–H); 3103 (ar. C–H); 2971, 2932, 2872 (C–H); 1746, 1713, 1645 (C=O); 1597, 1569, 1523 (C=C), (C=N), (N–H); 1472, 1437, 1393, 1373 (C–H); 1313, 1301, 1248, 1190, 1160, 1139, 1021 (C–O), (C–N), (C–O–C); 698 (ar. C–H) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$/TMS): δ = 9.14–9.13 (m, 1 H, H-7), 8.29–8.19 (m, 2 H, H-4, H-5), 6.74 (br. d, 1 H, N*H*), 4.81–4.78 (dd, $^3J_{9,NH}$ = 8.44 Hz, $^3J_{9,10}$ = 4.70 Hz, 1 H, H-9), 4.38–4.14 (m, 2 H, H-13), 4.04 (s, 3 H, H-1), 2.32 (dq, $^3J_{10,9}$ = 4.70 Hz, $^3J_{10,11/11'}$ = 6.91 Hz, 1 H, H-10), 1.33 (t, $^3J_{14,13}$ = 7.14 Hz, 3 H, H-14), 1.03, 1.01 (d, d, $^3J_{11/11',10}$ = 6.91 Hz, 6 H, H-11, H-11′) ppm. ^{13}C NMR (100 MHz, $CDCl_3$/TMS): δ = 175.56 (C-8), 164.92 (C-2), 164.64 (C-12), 149.99 (C-3), 148.41 (C-7), 136.20 (C-5), 132.53 (C-6), 124.87 (C-4), 61.69 (C-9), 57.74 (C-13), 53.20 (C-1), 31.38 (C-10), 19.01, 18.00 (C-11, C-11′), 14.30 (C-14) ppm. MS (70 eV, EI): *m/z* (%): 308 (5) ($M^{+\cdot}$), 235 (85) (M – $C_3H_5O_2$), 181 (32) (M – $C_7H_{11}O_2$), 164 (100) (M – $C_6H_{10}NO_3$), 78 (12) ($C_5H_4N^+$).

2MeO-5PheNHdipic (3d): Yield 54% (0.96 g). $C_{19}H_{20}N_2O_5$ (356.37): calcd. C 64.04, H 5.66, N 7.86; found C 63.88, H 5.66, N 7.74. IR (KBr) $\tilde{\nu}$ = 3321 (N–H); 3137, 3068, 3029 (ar. C–H); 2980, 2946, 2870 (C–H); 1734, 1647 (C=O); 1595, 1568, 1526, 1498 (C=C), (C=N), (N–H); 1455, 1439, 1375 (C–H); 1309, 1285, 1194, 1161, 1117, 1099, 1022 (C–O), (C–N), (C–O–C); 748, 700 (ar. C–H) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$/TMS): δ = 9.00–8.99 (m, 1 H, H-7), 8.21–8.16 (m, 2 H, H-4, H-5), 7.32–7.24 (m, 3 H, H-13, H-14, H-15), 7.15–7.12 (m, 2 H, H-12, H-16), 6.67 (br. d, 1 H, N*H*), 5.09–5.04 (dt, $^3J_{9,NH}$ = 7.49 Hz, $^3J_{9,10}$ = 5.93 Hz, 1 H, H-9), 4.25 (q, $^3J_{18,19}$ = 7.16 Hz, 2 H, H-18), 4.03 (s, 3 H, H-1), 3.34–3.23 (m, 2 H, H-10), 1.31 (t, $^3J_{19,18}$ = 7.16 Hz, 3 H, H-19) ppm. ^{13}C NMR (100 MHz, $CDCl_3$/TMS): δ = 171.27 (C-8), 164.87 (C-2), 164.15 (C-17), 150.01 (C-3), 148.14 (C-7), 136.26 (C-5), 135.49 (C-11), 132.34 (C-6), 129.28 (C-12, C-16), 128.74 (C-13, C-15), 127.40 (C-14), 124.91 (C-4), 61.96 (C-18), 53.66 (C-9), 53.21 (C-1), 37.70 (C-10), 14.15 (C-19) ppm. MS (70 eV, EI): *m/z* (%): 356 (6) ($M^{+\cdot}$), 283 (14) (M – $C_3H_5O_2$), 176 (97) (M – $C_{11}H_{16}O_2$), 164 (100) (M – $C_{11}H_{14}NO_2$), 148 (11) (M – $C_{12}H_{16}O_3$), 131 (18) (M – $C_{12}H_{19}NO_3$), 91 (19) ($C_7H_7^+$), 78 (12) ($C_5H_4N^+$).

Preparation of the Vanadium(IV) Compounds 4a–d and 5a–d. 4a–b: A solution of the ligand (1.0 mmol) and sodium acetate·$3H_2O$ (136 mg, 1.0 mmol) in water (10 mL) and THF (1 mL) was mixed with a solution of $VOSO_4$·$5H_2O$ (127 mg, 0.5 mmol) in water (2 mL) at 50 °C to yield light green solids. **4c–d**, **5a–d:** To a solution of the proligand (1.0 mmol) and sodium acetate·$3H_2O$ (136 mg, 1.0 mmol) in water (3 mL) and THF (6 mL) was added a solution of vanadyl sulfate·$5H_2O$ (127 mg, 0.5 mmol) in water (3 mL). The resulting green slurry was kept at reflux for ca. 6 h to yield a clear green solution. The solvent was evaporated, the residue was dissolved in THF and filtered. Evaporation to dryness yielded green solids.

[VO(5-*i*PrOdipic)$_2$] (4a): Yield 74% (185 mg). $C_{20}H_{20}N_2O_9V$·H_2O (501.34): calcd. C 47.91, H 4.42, N 5.59; found C 47.56, H 4.45, N 5.08. IR (KBr) $\tilde{\nu}$ = 3114, 3058 (ar C–H); 2983, 2873 (C–H); 1730 (C=O); 1687, 1652 $\nu_{as}(CO_2^-)$; 1609 (C=C), (C=N); 1484, 1397, 1353 (C–H), $\nu_s(CO_2^-)$; 1289 (C=C), (C=N); 1108, 1051 (C–O–C); 967 (V=O); 848 (ar. C–H), (C–CO_2); 750 (ar. C–H) cm^{-1}. MS (FAB): *m/z* = 484 (M – H_2O]. EPR (293 K, ethanol): g_0 = 1.975, A_0 = 93·10^{-4} cm^{-1}; (100 K, ethanol): $g_\perp$ = 1.985, $A_\perp$ = 60·10^{-4} cm^{-1}, g_{II} = 1.945, A_{II} = 167·10^{-4} cm^{-1}.

[VO(5-*s*BuOdipic)$_2$] (4b): Yield 65% (190 mg). $C_{22}H_{24}N_2O_9V$·THF (583.49): calcd. C 53.52, H 5.53, N 4.80; found C 52.70, H 5.47, N 4.98. IR (KBr) $\tilde{\nu}$ = 3116, 3080 (ar C–H); 2974, 2931, 2880, 2854 (C–H); 1727 (C=O); 1687 $\nu_{as}(CO_2^-)$; 1643, 1627, 1606, 1578 (C=C), (C=N); 1485, 1457, 1419, 1357 (C–H), $\nu_s(CO_2^-)$; 1283 (C=C), (C=N); 1130, 1108, 1046 (C–O–C); 977 (V=O); 851 (ar. C–H), (C–CO_2); 748 (ar. C–H) cm^{-1}. MS (FAB): *m/z* = 512 (M – THF]. EPR (100 K, ethanol): $g_\perp$ = 1.983, $A_\perp$ = 60·10^{-4} cm^{-1}, g_{II} = 1.945, A_{II} = 165·10^{-4} cm^{-1}.

[VO(5-GalOdipic)$_2$] (4c): Yield 84% (530 mg). $C_{38}H_{44}N_2O_{19}V$·4THF·0.75Na_2SO_4 (1260.64): calcd. C 51.45, H 6.08, N 2.22; found C 51.01, H 5.93, N 2.13. IR (KBr) $\tilde{\nu}$ = 3114, 3081 (ar C–H); 2988, 2936 (C–H); 1731 (C=O); 1681 $\nu_{as}(CO_2^-)$; 1642, 1608, 1578 (C=C), (C=N); 1457, 1437, 1415, 1383 (C–H), $\nu_s(CO_2^-)$; 1282, 1256, 1283, 1212 (C=C), (C=N); 1168, 1113, 1070, 1002 (O–CH); 973 (V=O); 898, 863 (ar. C–H), (C–CO_2); 747 (ar. C–H) cm^{-1}. MS (FAB): *m/z* = 884 (M + 1). EPR (293 K, THF): g_0 = 1.975, A_0 = 95·10^{-4} cm^{-1}; (100 K, ethanol): $g_\perp$ = 1.983, $A_\perp$ = 60·10^{-4} cm^{-1}, g_{II} = 1.945, A_{II} = 165·10^{-4} cm^{-1}.

[VO(5-InoOdipic)$_2$] (4d): Yield 85% (331 mg). $C_{28}H_{24}N_2O_{19}V$·$2H_2O$ (779.47): calcd. C 43.15, H 3.62, N 3.59; found C 43.06, H 4.17, N 3.65. IR (KBr) $\tilde{\nu}$ = 3075 (ar C–H); 2929, 2851 (C–H); 1724 (C=O); 1664 (sh) $\nu_{as}(CO_2^-)$; 1635, 1577 (C=C), (C=N); 1488, 1397, 1348 (C–H), $\nu_s(CO_2^-)$; 1287 (C=C), (C=N); 1162, 1124, 1090, 1048, 1006 (O–CH); 985 (V=O); 752 (ar. C–H) cm^{-1}. MS (FAB): *m/z* = 778 (M – 1]. EPR (293 K, THF): g_0 = 1.97, A_0 = 100·10^{-4} cm^{-1}; (100 K, water): $g_\perp$ = 1.983, $A_\perp$ = 60·10^{-4} cm^{-1}, g_{II} = 1.945, A_{II} = 165·10^{-4} cm^{-1}.

[VO(5-GlyOEtdipic)$_2$] (5a): Yield 73% (241 mg). $C_{22}H_{22}N_4O_{11}V$·THF·H_2O (659.50): calcd. C 47.35, H 4.89, N 8.50; found C 47.30, H 4.59, N 9.11. IR (KBr) $\tilde{\nu}$ = 3067 (ar C–H); 2981, 2953, 2873 (C–H); 1745 (C=O); 1670 $\nu_{as}(CO^{2-})$; 1597, 1542 (C=C), (C=N); 1437, 1417, 1377, 1353 (C–H), $\nu_s(CO_2^-)$; 1311, 1248, 1207 (C=C), (C=N); 1119, 1029 (O–CH); 975 (V=O); 873, 829 (ar. C–H), (C–CO_2); 749 (ar. C–H) cm^{-1}. MS (FAB): *m/z* = 570 (M), 592 [M + Na]. EPR (100 K, ethanol): $g_\perp$ = 1.99, $A_\perp$ = 60·10^{-4} cm^{-1}, g_{II} = 1.95, A_{II} = 170·10^{-4} cm^{-1}.

[VO(5-AlaOEtdipic)$_2$] (5b): Yield 65% (235 mg). $C_{24}H_{26}N_4O_{11}V$·THF·H_2O·0.25Na_2SO_4 (723.06): calcd. C 46.51, H 5.02, N 7.75; found C 46.15, H 4.45, N 7.99. IR (KBr) $\tilde{\nu}$ = 3070 (ar C–H); 2986, 2936, 2854 (C–H); 1738 (C=O); 1665 $\nu_{as}(CO_2^-)$; 1605, 1575, 1541 (C=C), (C=N); 1484, 1452, 1418, 1370, 1343 (C–H), $\nu_s(CO_2^-)$; 1284, 1212, 1179 (C=C), (C=N); 1123, 1047 (O–CH); 977 (V=O); 851 (ar. C–H), (C–CO_2); 749 (ar. C–H) cm^{-1}. MS (FAB): *m/z* = 598 (M), 620 [M + Na]. EPR (100 K, ethanol): $g_\perp$ = 1.985, $A_\perp$ = 60·10^{-4} cm^{-1}, g_{II} = 1.950, A_{II} = 163·10^{-4} cm^{-1}.

[VO(5-ValOEtdipic)$_2$] (5c): Yield 69% (228 mg). $C_{22}H_{22}N_4O_{11}V$·THF·H_2O (659.50): calcd. C 47.35, H 4.89, N 8.50; found C 47.30, H 4.59, N 9.11. IR (KBr) $\tilde{\nu}$ = 3065 (ar C–H); 2967, 2937, 2876 (C–H); 1739 (C=O); 1651 $\nu_{as}(CO_2^-)$; 1570, 1538 (C=C), (C=N); 1438, 1419, 1393, 1373 (C–H), $\nu_s(CO_2^-)$; 1312, 1249, 1197 (C=C), (C=N); 1157, 1021 (O–CH); 975 (V=O); 862, 823 (ar. C–H), (C–CO_2); 744, 697 (ar. C–H) cm^{-1}. MS (FAB): *m/z* = 654 (M + 1], 676 [M + Na]. EPR (100 K, ethanol): $g_\perp$ = 1.981, $A_\perp$ = 60·10^{-4} cm^{-1}, g_{II} = 1.945, A_{II} = 166·10^{-4} cm^{-1}.

[VO(5-PheOEtdipic)$_2$] (5d): Yield 50% (203 mg). $C_{36}H_{34}N_4O_{11}V$·0.5THF·$1.5H_2O$ (812.71): calcd. C 56.16, H 5.08, N 6.89; found C 56.35, H 5.19, N 6.78. IR (KBr) $\tilde{\nu}$ = 3061, 3030 (ar C–H); 2980, 2952, 2929, 2868 (CH); 1739 (C=O); 1665

$\nu_{as}(CO_2^-)$; 1602, 1536, 1497 (C=C), (C=N); 1455, 1438, 1374 (C–H), $\nu_s(CO_2^-)$; 1309, 1247, 1197, 1157 (C=C), (C=N); 1122, 1025 (O–CH); 966 (V=O); 855 (ar. C–H), (C–CO_2); 745, 700 (ar. C–H) cm^{-1}. MS (FAB): m/z = 750 (M + 1]. EPR (100 K, ethanol): $g_\perp$ = 1.983, $A_\perp = 60 \cdot 10^{-4}$ cm^{-1}, g_{II} = 1.945, $A_{II} = 165 \cdot 10^{-4}$ cm^{-1}.

Acknowledgments

This work was supported by the Hanseatic City of Hamburg, the Deutsche Forschungsgemeinschaft (grants RE 431/13-1 and /18-1) and the European Union (COST D21 0009/01).

[1] T. Kiss, T. Jakusch, *Metallotherapeutic Drugs and Metal-Based Diagnostic Agents: The Use of Metals in Medicine* (Eds.: M. Gielen, E. Tiekink), Wiley-VCH, Weinheim, **2005**, chapter 8.
[2] K. H. Thompson, C. Orvig, *J. Chem. Soc., Dalton Trans* **2000**, 2885–2892.
[3] a) K. H. Thompson, J. H. McNeill, C. Orvig, *Chem. Rev.* **1999**, *99*, 2561–2571; b) C. Orvig, K. H. Thompson, personal communication.
[4] S. Semiz, C. Orvig, J. H. McNeill, *Molec. Cell Biochem.* **2002**, *231*, 23–35.
[5] H. Sakurai, K. Fujii, H. Watanabe, H. Tamura, *Biochem. Biophys. Res. Commun.* **1995**, *214*, 1095–1101.
[6] D. C. Crans, L. Yang, T. Jakusch, T. Kiss, *Inorg. Chem.* **2000**, *39*, 4409–4416.
[7] H. Sakurai, Y. Kojima, Y. Yoshikawa, K. Kawabe, H. Yasui, *Coord. Chem. Rev.* **2002**, *226*, 187–198.
[8] J. Gätjens, B. Meier, T. Kiss, E. M. Nagy, P. Buglyó, H. Sakurai, K. Kawabe, D. Rehder, *Chem. Eur. J.* **2003**, *9*, 4924–4935.
[9] K. Isagawa, M. Kawai, Y. Fushizaki, *Nippon Kaguku Zasshi* **1967**, *88*, 553.
[10] K. Ohta, E. Kawachi, N. Inoue, H. Fukasawa, Y. Hashimoto, A. Itai, H. Kagechika, *Chem. Pharm. Bull.* **2000**, *48*, 1504–1513.
[11] H. W. Lee, Y. Kishi, *J. Org. Chem.* **1985**, *50*, 4402–4404.
[12] S. Ozaki, Y. Koga, L. Ling, Y. Watanabe, Y. Kimura, M. Hirata, *Bull. Chem. Soc. Jpn.* **1994**, *67*, 1058–1063.
[13] S. R. Tipson, *Methods Carbohydr. Chem.* **1963**, *2*, 246–250.
[14] M. Melchior, K. H. Thompson, J. M. Jong, S. J. Rettig, E. Shuter, V. G. Yuen, Y. Zhou, J. H. McNeill, C. Orvig, *Inorg. Chem.* **1999**, *38*, 2288–2293.
[15] K. Wieghardt, *Inorg. Chem.* **1978**, *17*, 57–64.
[16] D. Rehder, J. Costa Pessoa, C. F. G. C. Geraldes, M. M. C. A. Castro, T. Kabanos, T. Kiss, B. Meier, G. Micera, L. Pettersson, M. Rangel, A. Salifoglou, I. Turel, D. Wang, *J. Biol. Inorg. Chem.* **2002**, *7*, 384–396.

Received: February 15, 2006
Published Online: May 30, 2006

