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Iron Dienylphosphate Tricarbonyl Complexes as Water-Soluble Enzyme-Triggered CO-Releasing Molecules (ET-CORMs)

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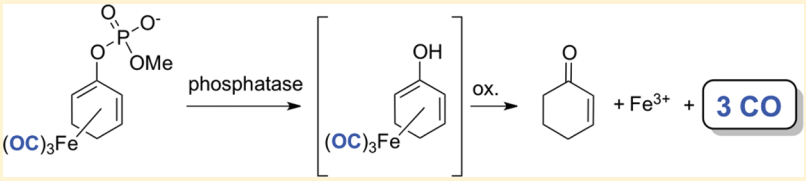
Supporting Information



ABSTRACT: A series of racemic phosphoryloxy-substituted (η^4-cyclohexadiene)$Fe(CO)_3$ complexes was synthesized by exploiting the O-phosphorylation of (dienol)$Fe(CO)_3$ intermediates generated in situ from the corresponding triisopropylsiloxy-protected complexes. The phosphorylated products were fully characterized by spectroscopic methods, including single-crystal X-ray diffraction in four cases. Monodeprotection of two dimethyl phosphate derivatives with trimethylamine led to the tetramethylammonium salts of the (cyclohexadienyl methyl phosphate)$Fe(CO)_3$ complexes. These compounds are the first water-soluble enzyme-trigged CO-releasing molecules (ET-CORMs). The phosphatase-induced CO release was monitored by means of GC. The biological activity was assessed in different cellular assays. The compounds were shown to be only slightly toxic, and a moderate anti-inflammatory potential was determined in an assay based on the inhibition of inducible NO synthase (iNOS)-induced NO production.

INTRODUCTION

While being a toxic gas, carbon monoxide (CO) has recently been found to induce several highly beneficial biological effects, such as cytoprotection, vasodilation, and inhibition of inflammation.[1] In this context, CO-releasing molecules (CORMs) have been developed as an attractive tool for CO delivery without the necessity of using CO gas.[2] The probably most prominent and intensely studied CORM is *fac*-[RuCl(glycinato)$(CO)_3$] (CORM-3), a water-soluble complex which releases CO under physiological conditions.[3] As a disadvantage, the half-life time of CORM-3 under physiological conditions is rather short, which makes it difficult to deliver controlled amounts of CO to a target tissue.[4] In recent years, some new transition-metal carbonyl complexes with promising CORM properties were developed.[5] However, a particularly important task remaining is the search for stable molecules, which only release CO after activation by means of a trigger.[6] In this context, UV-triggered CO release from certain transition-metal carbonyl complexes[7] and pH-dependent CO release from boranocarbonate (CORM-A1)[8] and amino derivatives thereof deserve mention.[9] In a novel and conceptually different approach, we recently introduced (acyloxybutadiene)$Fe(CO)_3$ complexes of type **1** as enzyme-triggered CO-releasing molecules (ET-CORMs).[10] On enzymatic ester cleavage (hydrolysis), these compounds are converted into highly labile dienol complexes (**2**), which in turn decompose under mildly oxidative conditions to give the free enone ligand (**3**) as well as three molecules of CO and a Fe^{3+} ion (Scheme 1). By investigating a series of complexes, we observed clear structure−activity relationships (SAR) and could identify some highly active compounds, using the inhibition of NO production by the pro-inflammatory protein inducible NO synthase (iNOS) as an indicator of the anti-inflammatory action of the released CO.[11]

For the possible application of ET-CORMs in medicine, the development of water-soluble compounds represented a relevant challenge. In this context, we envisioned that (phosphoryloxy diene)$Fe(CO)_3$ complexes such as **4** and **5**, respectively, might exhibit useful properties. As indicated in Scheme 2, we intended to synthesize such compounds by deprotection of suitable ester or amide precursors (**6**, **7**).

Racemic complexes of type **7** (substituted at the "inner" position) had been reported before by Okauchi et al. (and used in directed lithiation reactions), who prepared such compounds either through complexation of the corresponding dienes or by

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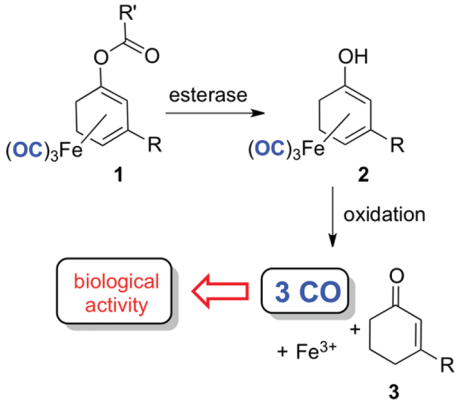
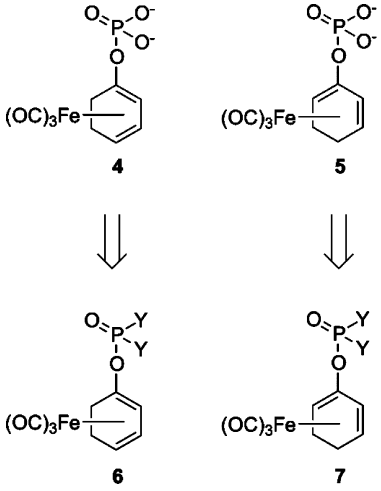
Scheme 1. Proposed Mechanism of Action Displayed by Enzyme-Triggered CO-Releasing Molecules (ET-CORMs) of Type 1



Scheme 2. Phosphate Complexes 4 and 5 and the Projected Precursors 6 and 7 (Y = NR_2, OR)

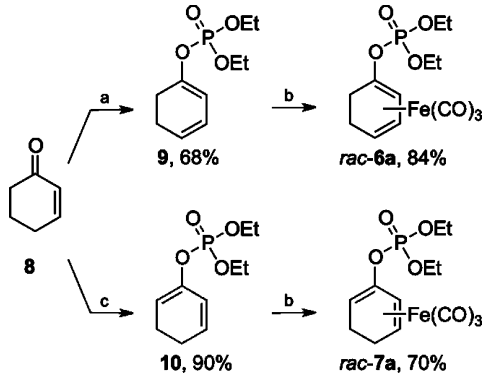


O-phosphorylation of an isolated (dienol)$Fe(CO)_3$ complex.[12] Here, we describe the synthesis of (dienylphosphate)$Fe(CO)_3$ complexes of both type **6** and type **7** using a different approach, which is based in both series on the phosphorylation of an in situ generated (dienol)$Fe(CO)_3$ intermediate. Moreover, we describe two water-soluble monodeprotected complexes, which were shown to act in the expected fashion: i.e., by releasing CO after activation by a phosphatase.

RESULTS AND DISCUSSION

The synthesis of cyclohexa-1,3-dien-1-yl diethyl phosphate (**9**) was achieved as shown in Scheme 3. In contrast to an earlier method (based on the deprotonation of nonconjugated 3-cyclohexen-1-one with lithium tetramethylpiperidide (LiTMP)[13] or lithium diisopropylamide (LDA)[14] and subsequent reaction with diethyl chlorophosphate), we succeeded in regioselectively generating the required dienolate starting from 2-cyclohexen-1-one (**8**) by deprotonation with lithium hexamethyldisilazide (LiHMDS) in the presence of tripyrrolidinophosphoric acid triamide (TPPA) as a cosolvent. Complexation of the dienylphosphate **9** under established conditions ((η^4-benzylideneacetone)$Fe(CO)_3$ in toluene)[12] yielded the desired complex *rac*-**6a** in good overall yield (Scheme 3).

Scheme 3. [a]



[a]Legend: (a) LiHMDS, TPPA, THF, −78 °C, then $(EtO)_2P(O)Cl$; (b) (benzylideneacetone)$Fe(CO)_3$, toluene, reflux, 16 h; (c) LDA, THF, −78 °C, then $(EtO)_2P(O)Cl$. LiHMDS = lithium hexamethyldisilazide.

The regioisomeric diethylphosphoryloxy substituted complex *rac*-**7** was also prepared from **8** following the same methodology, with the only difference being that the deprotonation of **8** was performed with LDA in THF (without a cosolvent).[13] Our attempts to apply a similar procedure to the preparation of the related (cyclohexa-1,5-dien-1-yl dibenzyl phosphate)Fe$(CO)_3$ failed. Moreover, as a conceptual drawback, this method would require the separate synthesis of a dienylphosphate ligand for all individual target complexes prior to the complexation (with a rather elaborate $Fe(CO)_3$-transfer reagent). For this reason it seemed attractive to search for a (diversity-oriented) method allowing a late variation of the phosphate unit, employing a stable complex as a key precursor. In the course of a previous study we had developed an efficient access to O-acylated (1,3-cyclohexadienol)- and (1,5-cyclohexadienol)$Fe(CO)_3$ complexes, using stable (triisopropylsiloxycyclohexadiene)$Fe(CO)_3$ complexes which were converted into the corresponding esters in a one-pot desilylation/O-acylation process.[11] Accordingly, a related protocol was developed for the synthesis of the desired phosphoryloxy compounds (Scheme 4).[15] As reported, the siloxydiene **11** was obtained from **8** with LDA and trimethylsilyl chloride (TMSCl).[10] Subsequent complexation afforded *rac*-**13**, which was deprotected by addition of tetra-*n*-butylammonium fluoride (TBAF). The in situ formed (crude) dienol complex was reacted with NaH and a chlorophosphate reagent. This way, the complexes *rac*-**7b**, *rac*-**7c**, *rac*-**7d**, and *rac*-**7e** were obtained with high yield and good reproducibility (Table 1). Starting from the triisopropylsilyl (TIPS)-based siloxydiene **12**, the resulting complex (*rac*-**14**) was sufficiently stable to be isolated and purified by chromatography (Scheme 4). Using pure *rac*-**14**, the yield of the phosphorylated products was clearly improved as demonstrated in the preparation of compounds *rac*-**7b** and *rac*-**7e**, respectively (Table 1). Moreover, the procedure proved to be more convenient from an operational point of view, as the isolated complex *rac*-**14** can be converted into a variety of different phosphates with little effort. The high price of TIPSOTf (as compared to TMSCl), however, makes it desirable to use the TMSCl-based protocol for the large-scale synthesis of particular target complexes.

The structural specifications of the differently protected phosphate complexes of type **7** prepared according to Scheme 4

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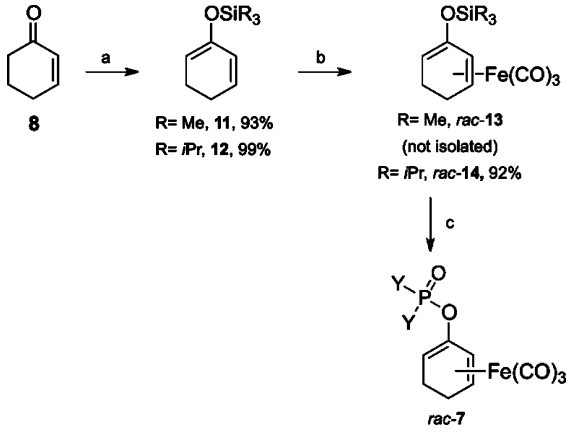
Scheme 4. [a]



[a]Legend: (a) LDA, THF, −78 °C, then TMSCl or TIPSOTf; (b) $Fe_2(CO)_9$, Et_2O, 40 °C, 16 h; (c) NaH, THF, TBAF, 0 °C, then $Y_2P(O)Cl$ (Y = OR, NR_2; for yields, see Table 1). TIPSOTf = triisopropylsilyl trifluoromethanesulfonate; TBAF = tetra-*n*-butylammonium fluoride.

Table 1. Complexes of Type 7 Synthesized According to Scheme 4[a]

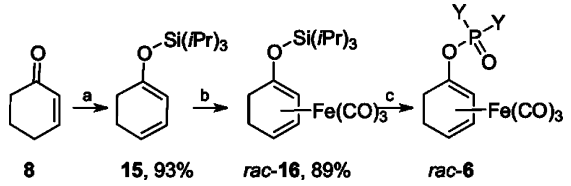
entry	R	Y	product	overall yield (%)
1	Me	OMe	*rac*-**7b**	40
2	*i*Pr	OMe	*rac*-**7b**	83
3	Me	OPh	*rac*-**7c**	60
4	Me	OBn	*rac*-**7d**	59
5	Me	NMe_2	*rac*-**7e**	60
6	*i*Pr	NMe_2	*rac*-**7e**	73

[a]Yields refer to the overall yield starting from cyclohexenone **8**.

(as potential substrates for the final decomplexation) and the yields obtained are summarized in Table 1.

The synthesis of the isomeric compounds of type **6** was achieved by applying a related methodology (Scheme 5).

Scheme 5. [a]



[a]Legend: (a) LiHMDS, TPPA, THF, −78 °C, then TIPSOTf; (b) $Fe_2(CO)_9$, Et_2O, 40 °C, 16 h; (c) NaH, THF, TBAF, 0 °C, then $R'_2P(O)Cl$. LiHMDS = lithium hexamethyldisilazide; TPPA = trispyrrolidinophosphoric acid triamide.

Deprotonation of **8** with LiHMDS in the presence of TPPA yielded the 1,3-cyclohexadienolate, which was protected (O-silylated) with TIPSOTf. Complexation of the resulting dienol ether **15** with $Fe_2(CO)_9$ gave *rac*-**16** in an excellent overall yield. Desilylation with TBAF in the presence of NaH and reaction of the resulting dienolate complex with an appropriate chlorophosphate reagent ($Y_2P(O)Cl$) yielded the desired complexes of type **6** in good overall yields from cyclohexenone **8** (Table 2).

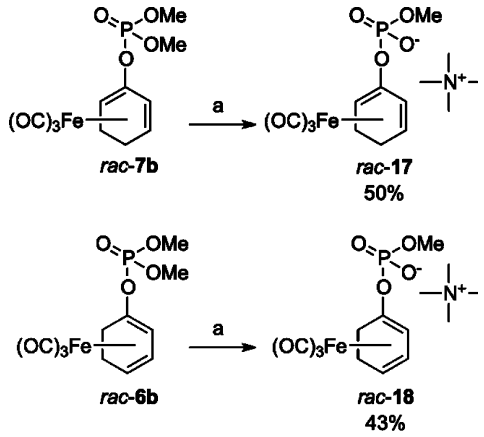
With a set of differently protected phosphate complexes of types **6** and **7** in our hands, we next investigated possibilities for

Table 2. Complexes of Type 6 Synthesized According to Scheme 5

entry	Y	product	yield (step c) (%)	overall yield (%)
1	OMe	*rac*-**6b**	87	72
2	OPh	*rac*-**6c**	83	69
3	OBn	*rac*-**6d**	60	50
4	NMe_2	*rac*-**6e**	51	42

their selective deprotection to compounds **4** and **5**, respectively. For this purpose, we tested a variety of different methods and rapidly recognized the difficulty of this task.[16] For instance, the attempted deprotection of *rac*-**7a** and *rac*-**7b** using TMSBr in CH_2Cl_2[17] only led to decomposition. While no conversion of the starting material was observed when we tried to reductively deprotect the diphenylphosphate complex *rac*-**7c** with PtO_2/H_2, the hydrogenation of the benzyl-protected derivative *rac*-**7d** in the presence of Pd/C resulted in the formation of the monodeprotected product. Also, the attempted deprotection of the phosphoramidate *rac*-**7e** under acidic conditions led to either decomposition or the formation of an unidentifiable side product. Finally, we succeeded in achieving at least a clean monodeprotection through nucleophilic cleavage of the dimethyl phosphates *rac*-**6b** and *rac*-**7b**. For this purpose, a solution of the complex in acetone was treated with an excess of an Me_3N (solution in EtOH) and the mixture was heated to 40 °C (Scheme 6).[18] After complete conversion (TLC control)

Scheme 6. [a]



[a]Legend: (a) NMe_3 (4.2 M in EtOH), 40 °C, acetone.

and removal of all volatiles, the resulting complexes were purified by reversed-phase column chromatography. Recrystallization from acetonitrile finally yielded the desired complexes *rac*-**17** and *rac*-**18**, respectively, as hygroscopic powders.

All compounds synthesized were fully characterized by the common spectroscopic methods (IR, NMR, MS), and the structures of *rac*-**6b**, *rac*-**6e**, *rac*-**7d**, and *rac*-**7e** were additionally confirmed by single-crystal X-ray diffraction analysis. Similar to the situation in (acyloxycyclohexadiene)$Fe(CO)_3$ complexes,[11,12,19] the $Fe(CO)_3$-complexed diene unit of the phosphoryloxy-substituted derivatives reported here is virtually planar and the cyclohexadiene ring adopts a boat-type conformation with torsion angles between 41.6(4) and 44.7(3)° for the endocyclic C_{sp^2}−C_{sp^3} bonds of *rac*-**6b**, *rac*-**7d**, and *rac*-**7e**. The torsion angles in each of these molecules do not differ more than 1.7(4)°, corresponding to an almost

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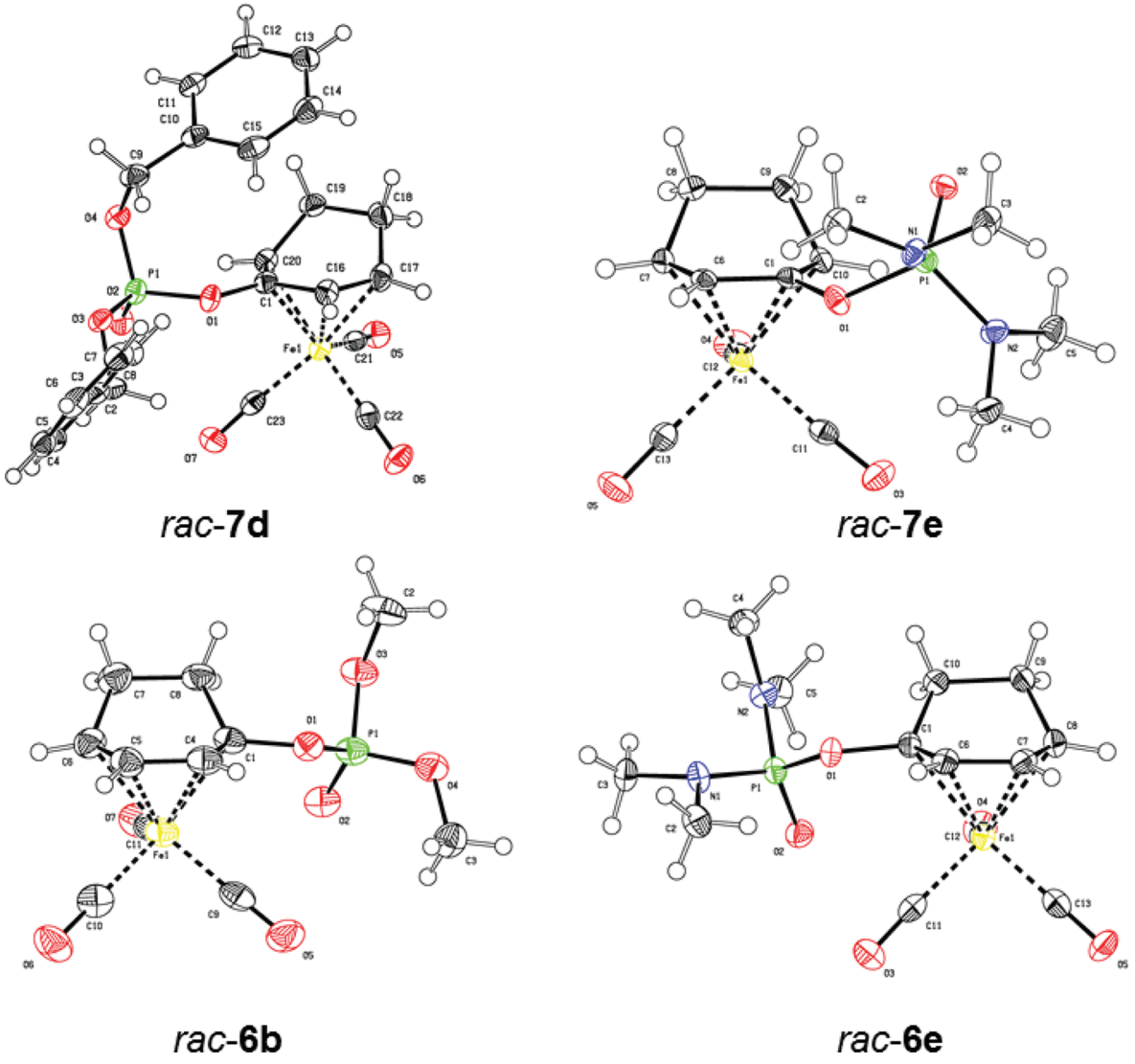

Figure 1. Molecular structures of *rac*-**6b**, *rac*-**6e**, *rac*-**7d**, and *rac*-**7e** in the crystalline state. Ellipsoids are drawn at the 50% probability level. Legend to colors: white, hydrogen; black/white, carbon; red, oxygen; yellow, iron; green, phosphorus; blue, nitrogen.

undistorted (symmetric) boat conformation of the cyclohexadiene ring. Only in complex *rac*-**6e** is the carbon skeleton somewhat distorted with torsion angles of 38.0(3) and 45.2(3) °. In all cases, the iron atom is coordinated to all of the four diene carbon atoms with bond lengths between 2.047(3) and 2.130(6) Å and the $Fe(CO)_3$ tripod takes a conformation with one CO ligand eclipsing the C_{sp^3}–C_{sp^3} single bond. The C–C and Fe–C bond lengths within the (diene)$Fe(CO)_3$ moiety fit in with those of related complexes found in the CSD database.[20] In comparison to noncomplexed dienes, the inner bond of the diene system is noticeably shorter while the C=C bonds are significantly elongated.[21] The molecular structures of complexes *rac*-**7d**, *rac*-**7e**, and *rac*-**6e** are depicted in Figure 1. For the X-ray crystallographic data and structural details see the Supporting Information.

The obtained monodeprotected (dienylphosphate)$Fe(CO)_3$ complexes *rac*-**17** and *rac*-**18** were obtained as hygroscopic powders which were found to be water-soluble (solubility >50 mM). To investigate their enzyme-triggered CO-releasing properties, complexes *rac*-**17** and *rac*-**18** were stirred at 37 °C with four different commercial phosphatases ((1) alkaline phosphatase from bovine intestinal mucosa, (2) alkaline phosphatase from porcine kidney, (3) acid phosphatase from potato, and (4) acid phosphatase from wheat germ) and without a phosphatase (control). The (phosphatase-triggered) CO release was then monitored by headspace GC. The detected CO release from *rac*-**17** and from *rac*-**18** is shown in Figure 2.

Without enzyme and in the presence of bovine or porcine alkaline phosphatases, no significant CO release was detected from *rac*-**17**. However, the plant phosphatases (from wheat germ or potato) clearly induced a CO release. The investigation of *rac*-**18** revealed the same trends, with the difference being that a slow CO release was observed in this case without enzyme as well as with the bovine or porcine enzymes (Table 3, Figure 2 bottom).

Compounds *rac*-**17** and *rac*-**18** released nearly 3 equiv of CO upon enzymatic activation (by wheat phosphatase). In contrast to the performance of the corresponding acetoxy compounds the CO release was very slow but also very effective. The comparison of the CO-release kinetics of *rac*-**17** and *rac*-**18** revealed that the CO release from the complex substituted at the "inner" position of the diene (*rac*-**17**) is faster than that from the "outer" substituted complex (*rac*-**18**). Remarkably,

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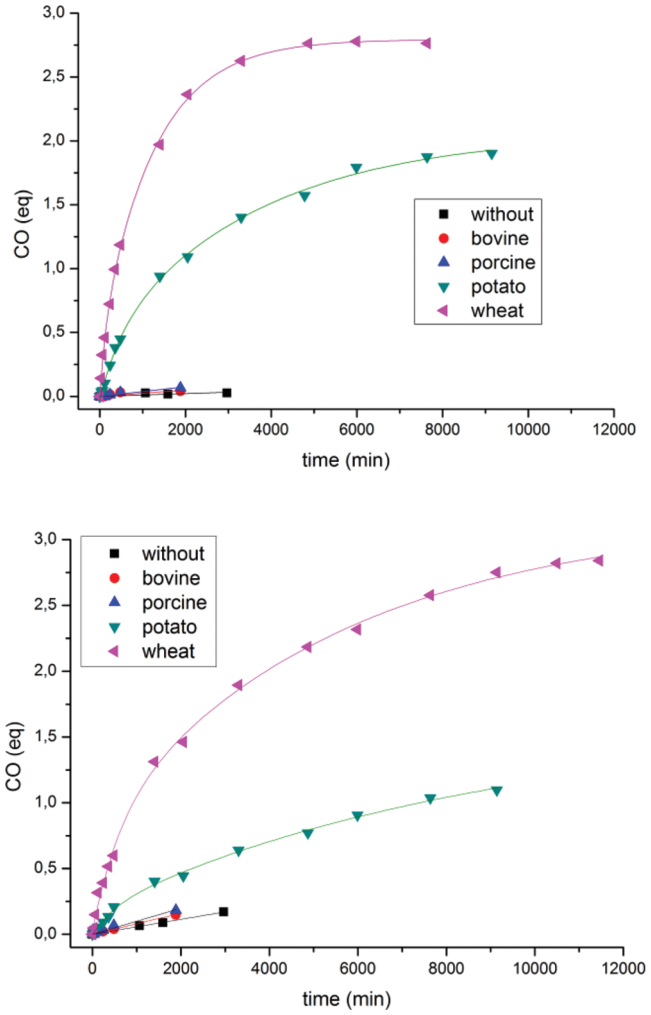

Figure 2. CO release from *rac*-**17** (top) and of *rac*-**18** (bottom) as a function of time: (black ■) without phosphatase; (red ●) alkaline phosphatase from bovine intestinal mucosa; (blue ▲) alkaline phosphatase from porcine kidney; (green ▼) acid phosphatase from potato; (pink ▲) acid phosphatase from wheat germ.

Table 3. CO Release from *rac*-17 and *rac*-18 Triggered by Different Phosphatases[a]

complex	enzyme[b]	amt of released CO (equiv)[b]	time (min)[b]	$t_{0.5}$ (min)
rac-**17**	none	0.03	2970	
rac-**17**	bovine	0.04	1884	
rac-**17**	porcine	0.07	1884	
rac-**17**	potato	1.90	9149	546
rac-**17**	wheat	2.78	5989	135
rac-**18**	none	0.17	2970	
rac-**18**	bovine	0.15	1884	
rac-**18**	porcine	0.18	1884	
rac-**18**	potato	1.09	9138	2250
rac-**18**	wheat	2.84	11456	333

[a]Shown are the monitoring time, the absolute amount of released CO, and the time needed for the release of 0.5 equiv of CO ($t_{0.5}$). [b]Legend: bovine, alkaline phosphatase from bovine intestinal mucosa; porcine, alkaline phosphatase from porcine kidney; potato, acid phosphatase from potato; wheat, acid phosphatase from wheat germ.

this behavior is different from that of the related acyloxy compounds. This suggests that the enzymatic hydrolysis is the rate-determining step of the CO-release mechanism, while the phosphate hydrolysis (at least with the particular enzymes used in this study) is clearly slower than the ester hydrolysis.

Having demonstrated the general ability of the water-soluble complexes *rac*-**17** and *rac*-**18** to act as phosphatase-triggered CO-releasing molecules, we next turned our attention to studying their biological activity using different cell lines. For this aim, cytotoxicity, inhibition of VCAM-1, and induction of HO-1 were assessed. Both compounds did not display any toxicity against human umbilical vein endothelial cells (HUVEC) or renal proximal tubule epithelial cells (PTEC). However, they were also not able to induce HO-1 or to inhibit TNF-α-mediated VCAM-1 expression (as observed for CORM-3).[22] The biological effects of the compounds were also assessed in the murine macrophage cell line RAW264.7 by determining their influence on (1) the cell viability (Figure 3, left graphs and Table 4) and (2) the lipopolysaccharide (LPS)-induced production of NO by iNOS (Figure 3, right graphs).

The inner and outer phosphate ester complexes *rac*-**17** and *rac*-**18** were found to differ significantly in their biological activity toward RAW264.7 cells according to the MTT and Griess assays. Whereas the inner isomer *rac*-**17** displays both an influence on the cell viability with an IC_{20} value of 252 ± 39 μM (an IC50 value could not be determined due to the overall low toxicity) and an inhibition of the LPS-induced NO production by 31% at 100 μM, the outer complex *rac*-**18** proved to be virtually inactive up to a concentration of 100 μM without displaying a significant toxicity. These observations are in agreement with the data found in the kinetic measurements, where a faster CO release was monitored for *rac*-**17** as compared to *rac*-**18** (Table 3).

■ CONCLUSIONS

In the search for enzyme-triggered CO-releasing molecules (ET-CORMs) with improved properties, we have studied phosphoryloxy-substituted (cyclohexadiene)$Fe(CO)_3$ complexes as potentially water-soluble compounds. While the deprotection of various phosphate derivatives proved difficult, we succeeded in obtaining the monodeprotected complexes *rac*-**17** and *rac*-**18** from the dimethyl phosphate precursors synthesized through a novel one-pot desilylation/phosphorylation protocol. These compounds not only exhibited reasonable water solubility but also showed the expected CO-releasing properties on activation by different commercial acid phosphatases. The fact that only the plant phosphatases were active should not be interpreted at this point, because only four commercial phosphatases were tested so far, and the results obtained with the RAW cell line indicate that there was at least one active phosphatase present. Both compounds (*rac*-**17** and *rac*-**18**) were not active in HUVEC or PTEC with respect to their ability to inhibit TNF-α mediated VCAM-1 induction or to induce HO-1 expression, possibly as a consequence of a lack of suitable phosphatases in these cells or due to limited cellular uptake. Nevertheless, *rac*-**17** induced a moderate inhibition of NO production in RAW264.7 cells. In comparison to the related (acyloxydiene)$Fe(CO)_3$ complexes (we had introduced as ET-CORMs before),[11] the decreased toxicity of the phosphate complexes and their ability to release nearly 3 equiv of CO are potential advantages. While the results disclosed herein can be regarded as a first proof of concept, future work will focus on the development of more active phosphoryloxydiene-based complexes. This might be achieved by variation of the diene ligand or by preparation of completely

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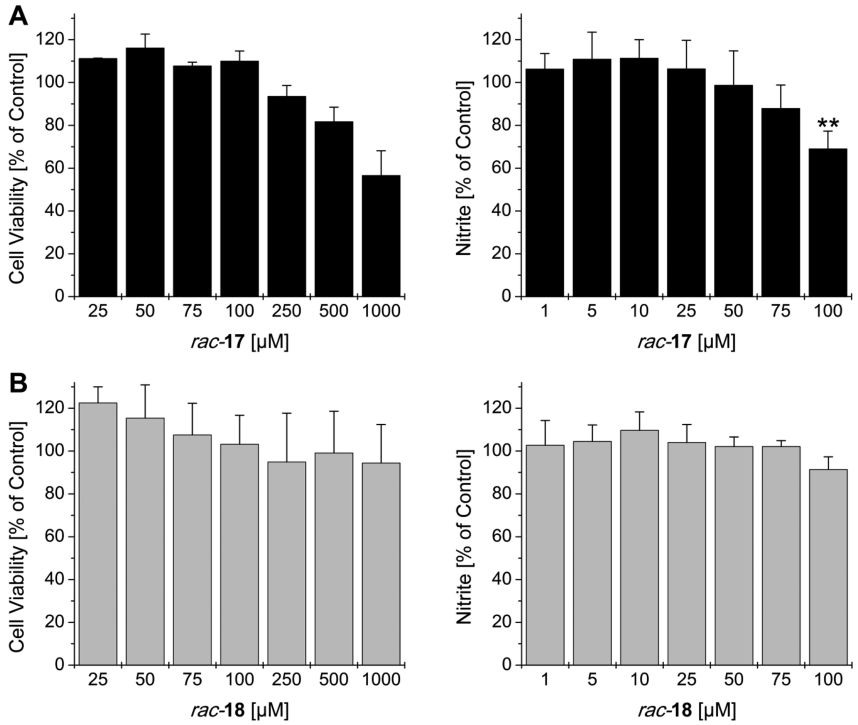

Figure 3. Results of the in vitro assays performed with RAW264.7 cells stimulated for 24 h with 10 ng/mL of LPS together with different concentrations of *rac*-**17** and *rac*-**18**. Charts on the left side refer to cell viability tests with MTT. Charts on the right display the influence of ET-CORMs on NO production (Griess assay). Data represent at least four independent experiments performed in quadruplicate. Levels of significance: (*) $p \leq 0.05$; (**) $p \leq 0.01$.

Table 4. Overview of Data Gained with LPS-Stimulated RAW264.7 Murine Macrophages[a]

compd	IC_{20} (μM)[b]	inhibition of NO production at 100 μM (%)
rac-**17**	252 ± 39	31
rac-**18**	>1000	*c*

[a]The IC_{20} value expresses a reduction of the cell viability of 20% as determined by the MTT assay. The inhibition of the NO production was measured with the Griess assay. [b]An IC_{50} value for *rac*-**17** could not be determined due to the overall low toxicity. [c]No statistically significant inhibition was found.

deprotected compounds such as **4** and **5**. Encouraged by the activity of *rac*-**17** in only one of three cell lines investigated, we consider the phosphatase-triggered compounds also as promising leads for the future development of cell-specific ET-CORMs, which are unknown so far.

EXPERIMENTAL SECTION

General Considerations. Unless otherwise stated, all ^1H and ^{13}C NMR spectra were recorded at room temperature in $CDCl_3$ on Bruker instruments (Avance DPX 300, Avance DRX 500, and Avance II 600). Chemical shifts (δ) are reported in parts per million (ppm) from tetramethylsilane using the residual solvent resonance as the internal standard ($CDCl_3$: 7.24 ppm for ^1H NMR, 77.0 ppm for ^{13}C NMR). Multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Ψ is added to indicate pseudomultiplicities. Multiplicities in ^{13}C spectra (according to P−C coupling) are not given. UV/vis spectra were recorded in 0.1 M phosphate buffer (pH 7.4) on a Beckmann Coultar DU 800 instrument (cell length 1 cm). IR spectra were recorded on a Perkin-Elmer Paragon 1000 FT-IR spectrometer in the ATR mode at room temperature. Relative intensities of the signals are given as strong (s), medium (m), weak (w), and broad (b). Direct inlet mass spectra (DIP-MS) were recorded at the Analytical Service Unit, University of Cologne, on Finnigan instruments (MAT Inocs 50 galaxy system (for EI LR-MS) and a MAT 900 (for HR-MS)). ESI-MS experiments were performed on a modified Thermo Fisher LTQ Orbitrap XL (Bremen, Germany) instrument (Figure 2). The spray voltage (3.4 kV), capillary voltage (3.0 V), tube lens voltage (3.0 V), and capillary temperature of 275 °C were applied as ESI conditions. To generate a stable electrospray, sheath gas and sweep gas were used (nitrogen 5.0, Linde, Germany, ≥99.999% N_2). An Agilent Technologies Model GC 6890N gas chromatograph coupled with an HP 5973N series mass selective detector and an HP 7683 GC autosampler was employed for all GC-MS analyses. Samples were separated on a 30 m × 0.25 mm HP-5 MS column. The column temperature was initially held at 50 °C for 2 min, and then the temperature was raised to 300 °C at a rate of 25 °C/min and held for 5 min. The total run time was 17 min. The injector temperature was maintained at 300 °C, and the injection volume was 1.0 μL in the split mode. Mass spectra were scanned from m/z 35 to 500. The electron impact ionization energy was 70 eV. Gas chromatograms for CO detection were recorded using a Varian CP-3800 gas chromatograph with helium as the carrier gas and a 3 m × 2 mm packed molecular sieve 13X 80-100 column. The gases were detected using a thermal conductivity detector (Varian) operated at 150 °C. CHN analyses were measured on an Elementar Vario EL machine. Melting points (uncorrected) were determined on a Büchi B-545 instrument and given in the form *T* (solvent from which the product was recrystallized). Analytical TLC was carried out using precoated silica gel plates (Merck TLC plates silica gel $60F_{254}$). Flash column chromatography was performed using silica gel (particle size 40−63 mM, Acros). All sensitive reactions were carried out in flame-dried glassware under an argon atmosphere. Analytical reversed-phase (RP) TLC was carried out using precoated silica gel plates (Merck TLC plates silica gel RP-18 $F_{254\ s}$). RP column chromatography was performed using a Grace Reveleris chromatography system with Receleris C18 flash cartridges (12 g, Grace). Chemicals were purchased from Merck, Sigma-Aldrich, Fluka, Acros, Lancaster, or Strem and used without further purification. Solvents were dried as follows: THF and Et_2O were distilled from sodium/benzophenone under an argon atmosphere, cyclohexenone was distilled and stored

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under argon, and diisopropylamine was refluxed over KOH and stored on KOH. Phosphatases were purchased from Sigma Aldrich as follows: phosphatase, alkaline from bovine intestinal mucosa, buffered aqueous solution, 2000−4000 DEA units/mg of protein (Sigma); phosphatase, acid from potato, lyophilized powder, 3.0−10.0 units/mg of solid (Sigma); phosphatase, acid from wheat germ, ≥0.4 unit/mg of solid (Sigma); phosphatase, alkaline from porcine intestinal mucosa, ≥1 unit/mg of solid (in glycine buffer) (Sigma).

Crystallographic Details. Measurements were made on a Nonius Kappa-CCD diffractometer with Mo Kα radiation (λ = 0.710 73 Å), and Denzo was used for data reduction. The structures were solved by direct methods (Shelxs97). Shelxl97 was used for full-matrix least-squares refinement on F^2. All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were placed in geometrically idealized positions and refined using the riding model. CCDC 883373−883376 contain the supplementary crystallographic data for this paper and can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif.

General Procedure for CO-Release Measurements in the Presence of Oxygen (GC). All reactions were performed in 10 mL headspace vials (BGB-analytics, Cat. No. 200410-F). Prior to the detection of the CO release a calibration was done. Calibrations were carried out with the solvent mixture (phosphate buffer (1 mL, (0.1 M, pH 7.4)) and DMSO (0.2 mL)) that was used for the CO-release experiments. For the CO calibration, the consumption of oxygen had to be included. For this purpose, the reaction vial was filled with the solvent mixture and completely degassed with nitrogen. Afterwards, 21% of the gas volume was replaced by oxygen to generate the composition of air (2.250 mL). To generate gas mixtures that are formed when a particular amount of O_2 is consumed in the course of the CO release, the volume of added oxygen was decreased by the amount that is theoretically needed for the release of the added CO (for a more detailed description see the Supporting Information). For the monitoring of the enzyme-triggered CO release, the particular complex (36 μmol) was dissolved in phosphate buffer (1.2 mL, (0.1 M, pH 7.4)). The particular enzyme (15 mg of PLE or 20 mg of LCR) was added and the vial was closed with a rubber vial cap. The reaction mixture was stirred at 37 °C. From time to time samples (50 μL) were taken and the CO release was quantified. The half-life times were determined directly from an exponential fit of the CO release (first or second order). The same procedure was repeated without enzyme for every complex, but there virtually was no CO release without enzyme.

Cells and Cell Culture. Murine macrophages, RAW 264.7, were grown in RPMI medium supplemented with 10% (v/v) heat inactivated fetal calf serum and 2 mM glutamine. Macrophages were cultured at 37 °C in humidified air containing 5% CO_2.

Human umbilical vein endothelial cells (HUVECs) were isolated from fresh umbilical cords as described previously.[23] The cells were grown in basal endothelial medium supplemented with 10% FBS and essential growth factors until they formed a confluent monolayer.

Human proximal tubular epithelial cells (PTECs) were obtained from surgical nephrectomy specimens following the methods described by Detrisac et al. The cells were cultured in serum-free Dulbeco's modified Eagle's medium (DMEM)/Ham's F12 medium (both from Seromed Bicochem, Berlin, Germany) in a 1:1 ratio. The medium was supplemented with insulin (5 μg/mL), transferrin (5 μg/mL), triiodothyronine (4 pg/mL), and epidermal growth factor (10 ng/mL) (all from Sigma, St. Louis, MO). After outgrowth of PTEC from explanted renal cortical remnants, which generally occurred within 7−10 days, cells were washed, trypsinized, and subcultured in tissue flasks.

Determination of Cell Viability by MTT Assay in RAW264. Cell viability was evaluated by determining the mitochondrial function of living cells on the basis of their ability to reduce a yellow dye, the tetrazolium salt 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), into violet formazan by mitochondrial dehydrogenases. Stock solutions of test compounds were prepared in EtOH (40 and 400 mM) and stored at −20 °C. Test concentrations were freshly prepared by diluting the stock solution in culture medium, and the final concentration of EtOH in the medium was ≤0.25%. Cells (5 × 10^3/well) were plated into 96-well plates and allowed to attach for 24 h. The test compounds were added to wells in several concentrations (100−1 and 1000−25 μM) in the presence and absence of LPS (10 ng/mL) and incubated for 20 h. The total assay volume was 100 μL. Ten microliters of a 4 mg/mL MTT solution in PBS was added to each well. After 4 h the culture medium was removed and 100 μL of a 10% SDS solution in water was put into each well to solubilize the formazan product. The absorbance was measured at 560 nm with a microplate reader (Multiskan Spectrum, Thermo) after 24 h incubation in the dark at room temperature. Every test was performed in quadruplicate, and all experiments have been repeated at least four times.

Determination of Cell Viability in HUVEC and PTEC. HUVEC and PTEC were cultured in 24-well plates until confluence. Thereafter the cells were cultured for 24 h in the presence of different concentrations (500−15 μM) of *rac*-**17** and *rac*-**18** by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) according to the manufacturer's instructions (Roche Diagnostics, Mannheim, Germany). Cell viability was also assessed by means of Trypan Blue exclusion. HUVEC or PTEC was cultured until confluence and thereafter was treated with 100 μM *rac*-**17** or *rac*-**18**. Cell viability was expressed as percent viable cells relative to the untreated cells. All experimental conditions were tested in triplicate in at least four different experiments.

Determination of Cell Proliferation by Crystal Violet Staining. Crystal violet was used to stain the nuclei of cells. The photometrically measured intensity of the dye directly correlates with the number of cells.[24]

In accord with the MTT assays, cells were seeded in 96-well plates at a density of 5 × 10^3 per well and cultured for 24 h. Then, the cells were incubated for another 24 h with medium supplemented with compounds and 10 ng/mL of LPS (*Escherichia coli* serotype 111:B4, Sigma). Controls received only culture medium with LPS or solvent and LPS, respectively.

Afterward, the supernatants were removed and cells were stained with 30 μL of crystal violet solution (0.5% crystal violet in 20% methanol) per well for 10 min. The crystal violet solution was removed, and then the cells were washed twice with 200 μL of water and air-dried overnight. Afterward, crystal violet was solubilized by addition of 100 μL of EtOH/Na citrate solution (EtOH + 0.1 M Na citrate, (v/v) 1/1) per well and the absorbance was determined at 560 nm.

Measurement of Nitrite Production by Griess Assay. To determine the anti-inflammatory activity of the compounds, the inhibition of a pro-inflammatory protein, i.e. iNOS (inducible NO synthase), was measured by determining nitrite accumulation in the culture medium as an indicator of nitric oxide production in the macrophages. For this the Griess method was used. RAW264.7 macrophages (8 × 10^4 cells/well) were plated in 96-well plates, allowed to attach for 24 h, and stimulated with LPS (10 ng/mL) in the presence or absence of the compounds to be tested (100−1 μM) for 24 h. As a negative control cells were incubated with test compounds only. Then, the culture medium was collected (50 μL/well), mixed with an equal volume of Griess reagent (0.1% NED (*N*-1-naphthylethylenediamine dihydrochloride), 1% sulfanilamine, 0.35% phosphoric acid in water), and incubated for 15 min at room temperature. The absorbance was measured at 560 nm. The nitrite content was then determined using sodium nitrite as a standard. Every test was performed in quadruplicate, and all experiments were repeated four times.

Western Blotting. HUVEC was harvested with trypsin/EDTA (Sigma-Aldrich, St. Louis, MO) and subsequently washed twice with cold PBS. The cells were lysed in lysis buffer (10 mM Tris, 2% SDS, 0.5% β-mercaptoethanol) (all from Sigma-Aldrich, St. Louis, MO). Protein concentrations were measured using Coomassie-Reagent (Pierce, Rockford, IL). Samples (20 μg of protein extract) were heated to 100 °C for 5 min, loaded, and separated on 10% SDS-polyacrylamide gels followed by semidry blotted onto PVDF membranes (Roche, Mannheim, Germany). The membrane was blocked with blocking buffer (5% w/v nonfat dry milk in TBS/Tween

0.5%) for 1 h at room temperature. Thereafter, the blot was incubated overnight at 4 °C, either with polyclonal anti-VCAM-1 (R&D Systems, Wiesbaden, Germany) or anti-HO-1 (Enzo Life Sciences, Lörrach, Germany). Subsequently the membrane was thoroughly washed with TBS-Tween and incubated for 1 h at room temperature with the appropriate horseradish peroxidase conjugated secondary antibody (Jackson ImmunoResearch, Baltimore, MD). Proteins were visualized using enhanced chemoluminescence technology according to the manufacturer's instructions (Pierce, Rockford, IL). To confirm equal protein loading, membranes were reprobed with monoclonal anti-GAPDH antibody (Abcam, Cambridge, U.K.).

Syntheses. *Synthesis of Phosphate Complexes of type rac-**6** and rac-**7**. General Procedures for the Desilylation/Phosphorylation Sequence. Procedure A (Starting from TMS-Protected Dienol **11**).* A solution of the silyloxydiene **11** (1.0 equiv) and $Fe_2(CO)_9$ (2.0 equiv) in Et_2O (concentration of the diene approximately 0.1 M) was heated to reflux for 16 h. After evaporation of the solvent, the residue was dissolved in THF (concentration of the theoretical amount of complex approximately 0.2 M) and NaH (1.3 equiv) was added. The mixture was cooled to 0 °C before a solution of TBAF in THF (1.3 equiv, 1.0 M in THF) was added. The reaction mixture was then stirred at 0 °C for approximately 10 min until deprotection was complete (TLC control). Subsequently, the chlorophosphate reagent (3.0 equiv) was added and the reaction mixture was warmed to room temperature and stirred for approximately 15 min (TLC control). Workup was initiated by addition of aqueous saturated NH_4Cl solution (20 mL), and the mixture was extracted with *M*tBE (3 × 30 mL). The combined organic phases were dried over $MgSO_4$, and the solvent was evaporated. The crude product was purified by column chromatography (CyHex/EtOAc) to yield the desired complex.

*Procedure B (Starting from rac-**14** or rac-**16**).* The reactions were performed on a 1.0−2.5 mmol scale. A flask was charged with NaH (1.3 equiv), and a solution of the (siloxydiene)$Fe(CO)_3$ complex (*rac*-**14** or *rac*-**16**) in THF (1.0 equiv, approximately 0.06 M) was added. The mixture was cooled to 0 °C before a solution of TBAF in THF (1.3 equiv, 1.0 M in THF) was added. The mixture was stirred at 0 °C for approximately 10 min until deprotection was complete (TLC control). Subsequently, the chlorophosphate reagent (3.0 equiv) was added and the mixture was warmed to room temperature and stirred for approximately 15 min (TLC control). Workup was initiated by addition of aqueous saturated NH_4Cl solution (20 mL), and the mixture was extracted with *M*tBE (3 × 30 mL). The combined organic phases were dried over $MgSO_4$, and the solvent was evaporated. The crude product was purified by column chromatography (CyHex/EtOAc) to yield the desired complex.

*(RS)-Tricarbonyl(η⁴-1-dimethylphosphoryloxy-1,3-cyclohexadiene)iron(0) (rac-**6b**).* Following general procedure B (chromatography with CyHex/EtOAc 2/1), complex *rac*-**6b** (759 mg, 87%) was obtained as a yellow oil that crystallized in the refrigerator after some days.

TLC: R_f (CyHex/EtOAc 1/1) = 0.25. ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.46 (d, J = 4.3 Hz, 1H, H2), 4.98 (Ψt, J = 5.5 Hz, 1H, H3), 3.78−3.68 (m, 6H, H7), 3.01−2.89 (m, 1H, H4), 2.24−2.11 (m, 1H, H6), 2.08−1.97 (m, 1H, H6), 1.86−1.74 (m, 1H, H5), 1.66−1.60 (m, 1H, H5). ^{13}C NMR (126 MHz, $CDCl_3$): δ 211.1 ($Fe(CO)_3$), 106.1 (C1), 79.4 (C2), 79.2 (C3), 59.5 (C4), 54.5 (C7), 29.0 (C6), 24.1 (C5). ^{31}P NMR (202 MHz, $CDCl_3$): δ −3.65. FT-IR (ATR): $\tilde{\nu}$ (cm^{-1}) 3003 (w), 2954 (m), 2853 (m), 2042 (s, ν ($Fe(CO)_3$)), 1961 (bs, ν ($Fe(CO)_3$)), 1456 (m), 1383 (m), 1329 (m), 1279 (s), 1183 (m), 1141 (s), 1122 (s), 1039 (s), 1003 (s), 929 (s), 893 (s), 849 (s), 776 (s), 698 (m), 642 (m), 611 (s). LR-MS (DIP-MS, 70 eV): m/z (%) 316 (3, $[M - CO]^+$), 288 (8, $[M - 2CO]^+$), 260 (18, $[M - 3CO]^+$), 258 (45), 228 (15), 203 (100), 181 (13), 167 (32), 151 (25), 134 (27), 127 (54), 109 (85), 94 (33), 84 (30), 79 (52), 78 (63), 77 (70), 56 (45). HR-MS (DIP-MS, 70 eV): m/z 315.978 (calcd ($[M - CO]^+$) m/z 315.9799). T_m (CH_2Cl_2) = 29.3 °C.

*(RS)-Tricarbonyl(η⁴-1-diphenylphosphoryloxy-1,3-cyclohexadiene)iron(0) (rac-**6c**).* Following general procedure A (chromatography with CyHex/EtOAc 5/1), complex *rac*-**6c** (489 mg, 83%) was obtained as a yellow oil.

TLC: R_f (CyHex/EtOAc 1/1) = 0.74. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41−7.33 (m, 5H, CH_{Ar}), 7.28−7.19 (m, 5H, CH_{Ar}), 5.52 (d, J = 4.4 Hz, 1H, H2), 5.13−4.97 (m, 1H, H3), 3.14−3.00 (m, 1H, H4), 2.37−2.23 (m, 1H, H6), 2.23−2.10 (m, 1H, H6), 1.90 (Ψddt, J = 15.2, 11.7, 3.5 Hz, 1H, H5), 1.75−1.65 (m, 1H, H5). ^{13}C NMR (101 MHz, $CDCl_3$): δ 210.7 ($Fe(CO)_3$), 150.5 (Cq_{Ar}), 129.8 (CH_{Ar}), 125.6 (CH_{Ar}), 120.2 (CH_{Ar}), 106.7 (C1), 79.6 (C2), 79.3 (C3), 59.6 (C4), 29.2 (C6), 24.2 (C5). ^{31}P NMR (162 MHz, $CDCl_3$): δ −16.73. FT-IR (ATR): $\tilde{\nu}$ (cm^{-1}) 3074 (w), 2946 (w), 2853 (w), 2045 (s, ν ($Fe(CO)_3$)), 1961 (bs, ν ($Fe(CO)_3$)), 1588 (m), 1486 (s), 1380 (m), 1294 (m), 1216 (m), 1183 (s), 1163 (s), 1140 (m), 1104 (s), 1065 (m), 1007 (s), 945 (s), 896 (s), 752 (s) 686 (s). LR-MS (DIP-MS, 70 eV): m/z (%) 412 (3, $[M - 2CO]^+$), 384 (5, $[M - 3CO]^+$), 326 (8), 305 (10), 212 (13), 169 (10), 119 (12), 94 (37), 77 (100), 65 (66), 56 (13), 51 (43). HR-MS (DIP-MS, 70 eV): m/z 384.020 (calcd ($[M - 3CO]^+$) m/z 384.021).

*(RS)-Tricarbonyl(η⁴-1-dibenzylphosphoryloxy-1,3-cyclohexadiene)iron(0) (rac-**6d**).* Following general procedure B (chromatography with CyHex/EtOAc 2/1), complex *rac*-**6d** (377 mg, 60%) was obtained as a yellow oil.

TLC: R_f (CyHex/EtOAc 1/1) = 0.63. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42−7.30 (m, 10H, CH_{Ar}), 5.52 (d, J = 4.1 Hz, 1H, H2), 5.16−4.98 (m, 5H, H7, H3), 3.04 (Ψs, 1H, H4), 2.24 (Ψdd, J = 18.8, 7.3 Hz, 1H, H6), 2.17−1.97 (m, 1H, H6), 1.87 (Ψtd, J = 11.8, 3.4 Hz, 1H, H5), 1.76−1.59 (m, 1H, H5). ^{13}C NMR (101 MHz, $CDCl_3$): δ 211.1 ($Fe(CO)_3$), 135.6 (Cq_{Ar}), 135.5 (Cq_{Ar}), 128.5 (CH_{Ar}), 128.0 (CH_{Ar}), 127.9 (CH_{Ar}), 106.4 (C1), 79.4 (C2/C3), 79.2 (C2/C3), 69.6 (C7), 69.4 (C7), 59.5 (C4), 29.1 (C6), 24.1 (C5). ^{31}P NMR (162 MHz, $CDCl_3$): δ −5.86. FT-IR (ATR): $\tilde{\nu}$ (cm^{-1}): 3020 (w), 2940 (w), 2884 (w), 2843 (w), 2043 (s, ν ($Fe(CO)_3$)), 1960 (bs, ν ($Fe(CO)_3$)), 1500 (w), 1454 (m), 1376 (m), 1325 (m), 1280 (m), 1213 (m), 1180 (w), 1141 (m), 1120 (m), 998 (s), 930 (m), 893 (s), 737 (m), 695 (s). LR-MS (DIP-MS, 70 eV): m/z (%) 440 (<1, $[M - 2CO]^+$), 412 (3, $[M - 3CO]^+$), 321 (8), 229 (3), 211 (2), 179 (3), 165 (3), 149 (5), 92 (8), 91 (100), 77 (12), 65 (21), 51 (11). HR-MS (DIP-MS, 70 eV): m/z 412.051 (calcd ($[M - 3CO]^+$) m/z 412.0527).

*(RS)-Tricarbonyl(η⁴-1-bis(dimethylamino)phosphoryloxy-1,3-cyclohexadiene)iron(0) (rac-**6e**).* Following general procedure B (chromatography with CyHex/EtOAc 2/1), complex *rac*-**6e** (120 mg, 51%) was obtained as a yellow oil that crystallized in the refrigerator after some days.

TLC: R_f (EtOAc) = 0.18. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.55 (d, J = 4.4 Hz, 1H, H2), 5.12−4.98 (m, 1H, H3), 2.99 (Ψdd, J = 3.9, 2.6 Hz, 1H, H4), 2.67, 2.65, 2.64, 2.63 (4 × s, 12H, H7), 2.19−2.08 (m, 2H, H6), 1.95−1.79 (m, 1H, H5), 1.77−1.63 (m, 1H, H5). ^{13}C NMR (101 MHz, $CDCl_3$): δ 211.8 ($Fe(CO)_3$), 107.0 (C1), 80.5 (C2), 78.9 (C3), 59.2 (C4), 36.4 (C7), 36.3 (C7), 36.3 (C7), 36.3 (C7), 29.5 (C6), 24.5 (C5). ^{31}P NMR (162 MHz, $CDCl_3$): δ 16.22. FT-IR (ATR): $\tilde{\nu}$ (cm^{-1}) 3446 (b w), 2943 (m), 2887 (m), 2841 (m), 2796 (w), 2036 (s, ν ($Fe(CO)_3$)), 1948 (bs, ν ($Fe(CO)_3$)), 1454 (m), 1382 (m), 1305 (m), 1224 (s), 1183 (m), 1138 (s), 1117 (s), 1066 (m), 977 (s), 851 (s), 754 (s), 708 (m), 608 (m). LR-MS (DIP-MS, 70 eV): m/z (%) 314 (7, $[M - 2CO]^+$), 286 (8, $[M - 3CO]^+$), 284 (20), 269 (5), 241 (7), 229 (10), 184 (12), 163 (15), 149 (17), 135 (100), 121 (21), 94 (25), 92 (33), 77 (55), 65 (28), 56 (25), 51 (27). HR-MS (DIP-MS, 70 eV): m/z 314.047 (calcd ($[M - 2CO]^+$) m/z 314.0482). T_m (CH_2Cl_2) = 43.7 °C.

*(RS)-Tricarbonyl(η⁴-1-dimethylphosphoryloxy-1,5-cyclohexadiene)iron(0) (rac-**7b**).* Following general procedure B (chromatography with CyHex/EtOAc 2/1) complex *rac*-**7b** (792 mg, 91%) was obtained as a yellow oil (procedure A afforded the same product in 43% yield).

TLC: R_f (CyHex/EtOAc 1/1) = 0.34. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.65 (d, J = 6.6 Hz, 1H, H6), 4.02−3.78 (m, 6H, H7), 3.58 (dd, J = 5.1, 2.7 Hz, 1H, H2), 2.93−2.73 (m, 1H, H5), 1.86−1.67 (m, 2H, H3), 1.63−1.41 (m, 2H, H4). ^{13}C NMR (101 MHz, $CDCl_3$): δ 210.4 ($Fe(CO)_3$), 129.8 (C1), 77.8 (C6), 56.9 (C2), 54.9 (C7), 51.2 (C5), 24.7 (C3), 23.1 (C4). ^{31}P NMR (162 MHz, $CDCl_3$): δ −3.64. FT-IR (ATR): $\tilde{\nu}$ (cm^{-1}): 3494 (b w), 3009 (w), 2956 (m), 2855 (m), 2042 (s, ν ($Fe(CO)_3$)), 1960 (bs, ν ($Fe(CO)_3$)), 1457 (s), 1427 (m),

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1393 (w), 1293 (s), 1256 (m), 1178 (s), 1119 (m), 1038 (s), 994 (m), 947 (s), 909 (s), 883 (m), 854 (s), 780 (m), 716 (w), 659 (w), 609 (s). LR-MS (DIP-MS, 70 eV) m/z (%) 316 (3, $[M - CO]^+$), 288 (11, $[M - 2CO]^+$), 260 (17, $[M - 3CO]^+$), 258 (53), 228 (20), 213 (8), 203 (43), 181 (17), 167 (25), 151 (39), 134 (28), 127 (45), 119 (33), 109 (100), 96 (26), 95 (31), 94 (65), 89 (65), 84 (37), 77 (73), 78 (74), 79 (74), 65 (70), 56 (52).

*(RS)-Tricarbonyl(η^4-1-diphenylphosphoryloxy-1,5-cyclohexadiene)iron(0) (rac-**7c**).* Following general procedure A (chromatography with CyHex/EtOAc 5/1), complex *rac*-**7c** (2.019 g, 65%) was obtained as a yellow oil.

TLC: R_f (CyHex/EtOAc 1/1) = 0.80. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41−7.33 (m, 4H, CH_{Ar}), 7.29−7.20 (m, 6H, CH_{Ar}), 5.64 (Ψdd, J = 14.4, 8.6 Hz, 1H, H6), 3.55 (dd, J = 5.3, 2.8 Hz, 1H, H2), 2.84−2.74 (m, 1H, H5), 1.74−1.67 (m, 2H, H3), 1.56−1.35 (m, 2H, H4). ^{13}C NMR (101 MHz, $CDCl_3$): δ 210.2 ($Fe(CO)_3$), 150.2 (Cq_{Ar}), 150.2 (Cq_{Ar}), 130.0 (CH_{Ar}), 1129.7 (C1), 25.8 (CH_{Ar}), 120.0 (CH_{Ar}), 78.5 (C6), 57.0 (C2), 51.2 (C5), 24.8 (C3), 23.1 (C4). ^{31}P NMR (162 MHz, $CDCl_3$): δ −16.88. FT-IR (ATR): $\tilde{\nu}$ (cm^{-1}) 3069 (w), 2941 (w), 2853 (w), 2046 (s, ν ($Fe(CO)_3$)), 1963 (bs, ν ($Fe(CO)_3$)), 1589 (w), 1487 (s), 1455 (s), 1427 (m), 1393 (w), 1299 (m), 1218 (m), 1161 (s), 1117 (m), 1072 (m), 1024 (m), 1009 (m), 959 (s), 908 (m), 856 (m), 769 (m), 754 (s), 687 (s), 608 (s). LR-MS (DIP-MS, 70 eV): m/z (%) 412 (2, $[M - 2CO]^+$), 384 (4, $[M - 3CO]^+$), 382 (5), 327 (8), 305 (11), 251 (6), 225 (5), 212 (11), 169 (7), 152 (8), 119 (13), 94 (40), 78 (25), 77 (100), 66 (18), 45 (45), 51 (38). HR-MS (DIP-MS, 70 eV): m/z 384.020 (calcd ($[M - 3CO]^+$) m/z 384.021).

*(RS)-Tricarbonyl(η^4-1-dibenzylphosphoryloxy-1,5-cyclohexadiene)iron(0) (rac-**7d**).* Following general procedure A (chromatography with CyHex/EtOAc 2/1), complex *rac*-**7d** (1.172 g, 52%) was obtained as a yellow oil that crystallized in the refrigerator after some days.

TLC: R_f (CyHex/EtOAc 5/1) = 0.16. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.50−7.33 (m, J = 2.7 Hz, 10H, CH_{Ar}), 5.52 (d, J = 6.3 Hz, 1H, H6), 5.24−5.03 (m, 4H, H7), 3.53 (Ψd, J = 1.4 Hz, 1H, H2), 2.90−2.69 (m, 1H, H5), 1.69−1.63 (m, 1H, H3), 1.63−1.55 (m, 1H, H3), 1.53−1.44 (m, 1H, H4), 1.42−1.33 (m, 1H, H4). ^{13}C NMR (126 MHz, $CDCl_3$): δ 210.4 ($Fe(CO)_3$), 135.1 (Cq_{Ar}), 135.1 (Cq_{Ar}), 129.7 (C1), 128.8 (CH_{Ar}), 128.6 (CH_{Ar}), 128.1 (CH_{Ar}), 77.9 (C6), 70.1 (C7), 56.9 (C2), 51.1 (C5), 24.6 (C3), 23.1 (C4). ^{31}P NMR (202 MHz, $CDCl_3$): δ −5.72. FT-IR (ATR): $\tilde{\nu}$ (cm^{-1}) 3036 (w), 3032 (w), 2946 (w), 2894 (w), 2852 (w), 2041 (s, ν ($Fe(CO)_3$)), 1953 (bs, ν ($Fe(CO)_3$)), 1496 (m), 1455 (s), 1426 (m), 1379 (m), 1276 (s), 1254 (m), 1213 (m), 1182 (s), 1119 (m), 1079 (m), 997 (s), 945 (s), 908 (s), 857 (s), 734 (s), 694 (s), 658 (m). LR-MS (DIP-MS, 70 eV): m/z (%) 440 (3, $[M - 2CO]^+$), 412 (5, $[M - 2CO]^+$), 321 (18), 265 (1), 242 (2), 229 (9), 211 (4), 149 (3), 119 (2), 92 (8), 91 (100), 77 (3), 65 (12), 56 (5). HR-MS (DIP-MS, 70 eV): m/z 412.052 (calcd ($[M]^+$) m/z 412.0527). T_m (CH_2Cl_2) = 40.0 °C.

*(RS)-Tricarbonyl(η^4-1-bis(dimethylamino)phosphoryloxy-1,5-cyclohexadiene)iron(0) (rac-**7e**).* Following general procedure B (chromatography with CyHex/EtOAc 2/1), complex *rac*-**7e** (2.00 g, 86%) was obtained as a yellow oil that crystallized in the refrigerator after some days (procedure A afforded the same product (2.02 g) in 65% yield).

TLC: R_f (EtOAc) = 0.25. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.63 (d, J = 6.3 Hz, 1H, H6), 3.52 (Ψd, J = 2.3 Hz, 1H, H2), 2.85−2.77 (m, 1H, H5), 2.71 (Ψt, J = 10.2 Hz, 12H, H7), 1.81−1.70 (m, 2H, H3), 1.56−1.43 (m, 2H, H4). ^{13}C NMR (101 MHz, $CDCl_3$): δ 211.0 ($Fe(CO)_3$), 130.3 (C1), 78.2 (C6), 57.8 (C2), 51.5 (C5), 36.5 (C7), 36.5 (C7), 36.5 (C7), 36.4 (C7), 24.8 (C3), 23.3 (C4). ^{31}P NMR (162 MHz, $CDCl_3$): δ 17.26. FT-IR (ATR): $\tilde{\nu}$ (cm^{-1}) 3506 (b w), 3006 (w), 2943 (m), 2895 (m), 2846 (m), 2806 (w), 2038 (s, ν ($Fe(CO)_3$)), 1953 (bs, ν ($Fe(CO)_3$)), 1457 (s), 1301 (s), 1233 (s), 1172 (s), 1119 (m), 1066 (m), 985 (s), 931 (m), 899 (s), 847 (s), 761 (s), 722 (m), 672 (m). LR-MS (DIP-MS, 70 eV): m/z (%) 342 (1, $[M - CO]^+$), 314 (7, $[M - 2CO]^+$), 286 (8, $[M - 3CO]^+$), 284 (22), 269 (5), 241 (7), 229 (6), 197 (4), 184 (6), 164 (11), 163 (13), 135 (100), 121 (16), 95 (7), 94 (16), 92 (31), 84 (15), 77 (45), 65 (15), 56 (20). HR-MS (DIP-MS, 70 eV): m/z 314.048 (calcd ($[M - 2CO]^+$) m/z 314.0482). T_m (CH_2Cl_2) = 30.3 °C.

Synthesis of the Monodeprotected Phosphate Complexes *rac*-17 and *rac*-18. General Procedure for Nucleophilic Monodeprotection. The dimethyl phosphate complex *rac*-**6b** or *rac*-**7b** (500 mg, 1.45 mmol, 1.0 equiv) was dissolved in acetone (50 mL), and trimethylamine (3.5 mL, 14.5 mmol, 10 equiv, 4.2 M in ethanol) was added dropwise. The solution was stirred at 40 °C, and the conversion was monitored by RP-TLC. In the case of incomplete conversion after 3 h, an additional 3.5 mL of trimethylamine was added and stirring was continued. After complete conversion the solvent was evaporated. The raw product was purified by column chromatography (RP-silica, MeCN/MeOH 9/1) and recrystallized from MeCN to give the pure ammonium phosphates as slightly yellow hygroscopic powders.

*(RS)-Tricarbonyl(η^4-1-tetramethylammoniomethylphosphoryloxy-1,5-cyclohexadiene)iron(0) (rac-**17**).* Yield: 452 mg (1.12 mmol, 77%). RP-TLC: R_f (MeCN/MeOH = 5:2) = 0.34. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.68 (d, J = 5.9 Hz, 1H, H6), 3.64 (Ψd, J = 10.5 Hz, 4H, H7, H2), 3.41 (s, 12H, $N(CH_3)_4^+$), 2.79 (Ψs, 1H, H5), 1.73 (Ψs, 2H, H3), 1.57−1.42 (m, 2H, H4). ^{13}C NMR (126 MHz, $CDCl_3$): δ 211.9 ($Fe(CO)_3$), 132.8 (C1), 77.8 (C6), 57.4 (C2), 55.8 ($N(CH_3)_4^+$), 53.0 (C7), 51.7 (C5), 24.9 (C3), 23.4 (C4). ^{31}P NMR (202 MHz, $CDCl_3$): δ −3.65. FT-IR (ATR): $\tilde{\nu}$ (cm^{-1}) 3376 (b m), 3030 (w), 2948 (w), 2846 (w), 2037 (s, ν ($Fe(CO)_3$)), 1949 (bs, ν ($Fe(CO)_3$)), 1646 (w), 1486 (m), 1461 (m), 1415 (w), 1254 (s), 1196 (m), 1174 (m), 1091 (s), 1045 (s), 949 (m), 899 (m), 871 (m), 830 (m), 775 (m), 610 (s). HR-MS (ESI-MS, 70 eV): m/z 328.951 (calcd ($[M]^-$) m/z 328.9508), 272.962 (calcd $[M - 2CO]^-$) m/z 272.9610, 242.951 (calcd $[C_7H_8O_4FeP]^-$ m/z 242.9504), 187.016 (calcd $[C_7H_8O_4P]^-$ m/z 187.0155).

*(RS)-Tricarbonyl(η^4-1-tetramethylammoniomethylphosphoryloxy-1,3-cyclohexadiene)iron(0) (rac-**18**).* Yield: 400 mg (0.99 mmol, 69%). RP-TLC: R_f (MeCN/MeOH 5/2) = 0.36. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.72 (d, J = 3.7 Hz, 1H, H2), 5.05−4.99 (m, 1H, H3), 3.69−3.54 (m, 3H, H7), 3.41 (s, 12H, $N(CH_3)_4^+$), 2.95 (Ψs, 1H, H4), 2.38−2.12 (m, 2H, H6), 1.95−1.76 (m, 1H, H5), 1.76−1.60 (m, 1H, H5). ^{13}C NMR (126 MHz, $CDCl_3$): δ 213.0 ($Fe(CO)_3$), 110.1 (C1), 80.1 (C2), 7.8 (C3), 58.7 (C4), 55.8 ($N(CH_3)_4^+$), 52.7 (C7), 29.4 (C6), 24.3 (C5). ^{31}P NMR (202 MHz, $CDCl_3$): δ −3.74. FT-IR (ATR): $\tilde{\nu}$ (cm^{-1}) 3366 (b m), 3030 (w), 2928 (w), 2846 (w), 2033 (s, ν ($Fe(CO)_3$)), 1947 (bs, ν ($Fe(CO)_3$)), 1650 (w), 1488 (m), 1382 (m), 1328 (m), 1242 (s), 1184 (m), 1154 (m), 1082 (s), 1045 (s), 1004 (m), 950 (m), 830 (m), 769 (m), 691 (m), 637 (m), 613 (s). HR-MS (ESI-MS, 70 eV): m/z 328.952 (calcd $[M]^-$ m/z 328.9508), 272.962 (calcd $[M - 2CO]^-$ m/z 272.9610), 242.951 (calcd $[C_7H_8O_4FeP]^-$ m/z 242.9504), 187.016 (calcd $[C_7H_8O_4P]^-$ m/z 187.0155).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

CIF files, text, tables, and figures giving crystallographic data, procedures, and analytical data of all synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) For a review, see: Ryter, S. W.; Alam, J.; Choi, A. M. K. *Physiol. Rev.* **2006**, *86*, 583−650.

(2) (a) Motterlini, R.; Otterbein, L. E. *Nat. Rev. Drug Discov.* **2010**, *9*, 728−743. (b) Romão, C. C.; Blättler, W. A.; Seixasa, J. D.; Bernardes, G. J. L. *Chem. Soc. Rev.* **2012**, *41*, 3571−3583.

(3) Naughton, P.; Shurey, S.; Green, C. J.; Johnson, T. R.; Mann, B. E.; Foresti, R.; Motterlini, R.; Clark, J. E. *Circ. Res.* **2003**, *93*, E2−E8.

(4) Motterlini, R.; Mann, B. E.; Foresti, R. *Expert. Opin. Investig. Drugs* **2005**, *14*, 1305−1318.

(5) Iron-containing CORMs: (a) Scapens, D.; Adams, H.; Johnson, T. R.; Mann, B. E.; Sawle, P.; Aqil, R.; Perrior, T.; Motterlini, R. *Dalton Trans.* **2007**, 4962−4973. (b) Fairlamb, I. J. S.; Lynam, J. M.; Moulton, B. E.; Taylor, I. E.; Duhme-Klair, A. K.; Sawle, P.; Motterlini, R. *Dalton Trans.* **2007**, 3603−3605. (c) Jackson, C. S.; Schmitt, S.; Dou, Q. P.; Kodanko, J. J. *Inorg. Chem.* **2011**, *50*, 5336−5338. (d) Hewison, L.; Crook, S. H.; Johnson, T. R.; Mann, B. E.; Adams, H.; Plant, S. E.; Sawle, P.; Motterlini, R. *Dalton Trans.* **2010**, *39*, 8967−8975. (e) Kretschmer, R.; Gessner, G.; Görls, H.; Heinemann, S. H.; Westerhausen, M. *J. Inorg. Biochem.* **2011**, *105*, 6−9. (f) Gonzalez, M. A.; Fry, N. L.; Burt, R.; Davda, R.; Hobbs, A.; Mascharak, P. K. *Inorg. Chem.* **2011**, *50*, 3127. (g) Hewison, L.; Johnson, T. R.; Mann, B. E.; Meijer, A. J. H. M.; Sawle, P.; Motterlini, R. *Dalton Trans.* **2011**, *40*, 8328−8334. CORMs based on other transition metals: (h) Atkin, A. J.; Williams, S.; Sawle, P.; Motterlini, R.; Lynam, J. M.; Fairlamb, I. A. S. *Dalton Trans.* **2009**, 3653−3656. (i) Zobi, F.; Degonda, A.; Schnaub, M. C.; Bogonda, A. Y. *Inorg. Chem.* **2010**, *49*, 7313−7322. (j) Zobi, F.; Blacque, O. *Dalton Trans.* **2011**, *40*, 4994−5001. (k) Crook, S. H.; Mann, B. E.; Meijer, A. J. H. M.; Adams, H.; Scapens, P. D.; Motterlini, R. *Dalton Trans.* **2011**, *40*, 4230−4235. (l) Bikiel, D. E.; Di Salvo, E. G. F.; Milagre, H. M. S.; Eberlin, M. N.; Correa, R. S.; Ellena, J.; Estrin, D. A.; Doctorovich, F. *Inorg. Chem.* **2011**, *50*, 2334−2345. (m) Brückmann, N. E.; Wahl, M.; Reiß, G. J.; Kohns, M.; Wätjen, W.; Kunz, P. C. *Eur. J. Inorg. Chem.* **2011**, 4571−4577. (n) Mohr, F.; Niesel, J.; Schatzschneider, U.; Lehmann, C. W. *Z. Anorg. Allg. Chem.* **2012**, *638*, 543−546. (o) Berends, H.-M.; Kurz, P. *Inorg. Chim. Acta* **2012**, *380*, 141−147. (p) Velasquez, V. P. L.; Jazzazi, T. M. A.; Malassa, A.; Görls, H.; Gessner, G.; Heinemann, S. H.; Westerhausen, M. *Eur. J. Inorg. Chem.* **2012**, 1072−1078.

(6) Foresti, R.; Bani-Hani, M. G.; Motterlini, R. *Intensive Care Med.* **2008**, *34*, 649−658. See also ref 2b.

(7) (a) Niesel, J.; Pinto, A.; Peindy N'Dongo, H. W.; Merz, K.; Ott, I.; Gust, R.; Schatzschneider, U. *Chem. Commun.* **2008**, 1798−1800. (b) Pfeiffer, H.; Rojas, A.; Niesel, J.; Schatzschneider, U. *Dalton Trans.* **2009**, 4292−4298. (c) Rimmer, R. D.; Richter, H.; Ford, P. C. *Inorg. Chem.* **2010**, *49*, 1180−1185. (d) Schatzschneider, U. *Inorg. Chim. Acta* **2011**, *374*, 19−23. (e) Dördelmann, G.; Pfeiffer, H.; Birkner, A.; Schatzschneider, U. *Inorg. Chem.* **2011**, *50*, 4362−4367. (f) Zhang, W.-Q.; Atkin, A. J.; Fairlamb, I. J. S.; Whitwood, A. C.; Lynam, J. M. *Organometallics* **2011**, *30*, 4643−4654.

(8) Motterlini, R.; Sawle, P.; Hammad, J.; Bains, S.; Alberto, R.; Foresti, R.; Green, C. J. *FASEB* **2005**, *19*, 284−286.

(9) Pitchumony, T. S.; Spingler, B.; Motterlini, R.; Alberto, R. *Org. Biomol. Chem.* **2010**, *8*, 4849−4854.

(10) Romanski, S.; Kraus, B.; Schatzschneider, U.; Neudörfl, J.-M.; Amslinger, S.; Schmalz, H.-G. *Angew. Chem., Int. Ed.* **2011**, *50*, 2392−2396.

(11) Romanski, S.; Kraus, B.; Guttentag, M.; Schlundt, W.; Rücker, H.; Adler, A.; Neudörfl, J.-M.; Alberto, R.; Amslinger, S.; Schmalz, H.-G. *Dalton Trans.* **2012**, *41*, in press.

(12) (a) Okauchi, T.; Teshima, T.; Hayashi, K.; Suetsugu, N.; Minami, T. *J. Am. Chem. Soc.* **2001**, *123*, 12117−12118. (b) Okauchi, T.; Teshima, T.; Sadoshima, M.; Kawakubo, H.; Kagimoto, K.; Sugahara, Y.; Kitamura, M. *Chem. Commun.* **2010**, *46*, 5015−5017.

(13) Blotny, G.; Pollack, R. M. *Synthesis* **1988**, 109−111.

(14) Dorn, V. B.; Badajoz, M. A.; Lockhart, M. T.; Chopa, A. B.; Pierini, A. B. *J. Organomet. Chem.* **2008**, *693*, 2458−2462.

(15) In comparison to the original procedure the solvent had to be changed from DMF to THF (to avoid a reaction of DMF with the chlorophosphate to give a Vilsmeier-type intermediate).

(16) For the TMSBr deprotection different temperatures and solvents were tried. For the hydrogenations different solvents and pressures from 1 atm to 50 bar were tried. Acidic deprotection of the phosphoramidates was tried under various conditions: e.g. 1−20% TFA in MeCN/H_2O, up to 4/1 TFA/H_2O or HCl/dioxane.

(17) For the deprotection of the noncomplexed dienyl dimethyl phosphate, see ref 13.

(18) For a similar method using aqueous MeN_3, see: Jentzsch, R.; Schneider, P.; Fischer, G. W. *J. Prakt. Chem.* **1975**, *317*, 721−732.

(19) Romanski, S.; Neudörfl, J.-M.; Schmalz, H.-G. *Acta Crystallogr.* **2011**, *E67*, m1530.

(20) Allen, F. R. *Acta Crystallogr.* **2002**, *B58*, 380−388.

(21) For a qualitative rationalization of this phenomenon, see: Deeming, A. J. Mononuclear Iron compounds with η^2- η^6 hydrocarbon ligands. In *Comprehensive Organometallic Chemistry*; Wilkinson, G., Stone, F. G. A., Abel, E. W., Eds.; Pergamon: Oxford, U.K., 1982; Vol. 4, p 377.

(22) Song, H.; Bergstrasser, C.; Rafat, N.; Höger, S.; Schmidt, M.; Endres, N.; Göbeler, M.; Hillebrands, J. L.; Brigelius-Flohé, R.; Banning, A.; Beck, G.; Lösel, R.; Yard, B. A. *Br. J. Pharmacol.* **2009**, *157*, 769−780.

(23) Yard, B.; Beck, G.; Schnuelle, P.; Braun, C.; Schaub, M.; Bechtler, M.; Göttmann, U.; Xiao, Y.; Breedijk, A.; Wandschneider, S.; Lösel, R.; Sponer, G.; Wehling, M.; van der Woude, F. J. *Am. J. Transplant.* **2004**, *4*, 22−30.

(24) Gillies, R.; Didier, N.; Denton, M. *Anal. Biochem.* **1986**, *159*, 109−113.