chemosensors

MDPI

Communication

An Organophosphorus(III)-Selective Chemodosimeter for the Ratiometric Electrochemical Detection of Phosphines

Sam A. Spring, Sean Goggins * and Christopher G. Frost

Department of Chemistry, University of Bath, Claverton Down, Bath BA2 7AY, UK; s.a.spring@bath.ac.uk (S.A.S.); c.g.frost@bath.ac.uk (C.G.F.)

* Correspondence: s.goggins@bath.ac.uk; Tel.: +44(0)1225-386231; Fax: +44(0)1225-386142

Received: 10 March 2019; Accepted: 3 April 2019; Published: 11 April 2019

Abstract: The high toxicity of phosphine and the use of organophosphines as nerve agent precursors has provoked the requirement for a rapid and reliable detection methodology for their detection. Herein, we demonstrate that a ferrocene-derived molecular probe, armed with an azidobenzene trigger, delivers a ratiometric electrochemical signal selectively in response to organophosphorus(III) compounds and can be accurately measured with an inexpensive, handheld potentiostat. Through an intensive assay optimization process, conditions were found that could determine the presence of a model organophosphine(III) nerve agent precursor within minutes and achieved a limit of detection for triphenylphosphine of just 13 ppm. Due to the portability of the detection system and the excellent stability of the probe in solution, we envisaged that this proof-of-concept of work could easily be taken into the field to enable potentially toxic organophosphorus(III) compounds to be detected at the point-of-need.

Keywords: phosphine detection; ratiometric sensing; electrochemical chemodosimeter; point-of-use

1. Introduction

Phosphine, the simplest phosphorus(III) compound, is a volatile toxic gas that is utilized in the agricultural industry as a fumigant [1,2]. Alkylphosphines and alkylphosphites also have acute toxicologies similar to phosphine [3–5]. Alkylphosphinites are listed as Schedule 1 compounds under the Chemical Warfare Convention as restricted precursors for the nerve agent VX, and alkylphosphites are listed as Schedule 3. In addition, phosphorus(III) compounds have been widely used in organic synthesis and the pharmaceutical industry, where a diverse number of new phosphorus(III) ligands with unknown toxicologies have been used in cross-coupling reactions, which remain a predominant synthetic tool [6,7]. However, though the toxicology of triphenylphosphine has been studied, with a permitted daily exposure (PDE) of 250 μg reported recently [5], only a few studies have explored the toxicity of less common aryl- and alkylphosphine ligands. In the absence of accurate toxicology, there remains a requirement to detect low-level concentrations of these potentially toxic analytes.

The detection of phosphorus(III) compounds can be performed using chemiluminescence [8], gas chromatography–mass spectrometry [9], and flame photometry [10]. However, these methodologies are limited by the requirement for sensitive, lab-based equipment, and the transportation of highly toxic samples to them. Extending the current methods to organophosphorus(III) species has so far been limited to just volatile alkylphosphines until very recently, when nerve-agent mimics were shown to be detected via fluorescence [11]. However, for their adoption into a point-of-care device, the construction of a lightbox is needed [12].

Electrochemistry has emerged as a popular detection method, due to its rapid sample detection rate, and its use of inexpensive equipment, which allows for applications within small,

portable detection systems [13,14]. To combat problems arising from miniaturization, ratiometric electrochemical probes have become more widespread, with the minimization of errors increasing their reliability and reproducibility [15,16]. By obtaining direct conversions from the sample, systematic and sampling errors are reduced. Ferrocene-based probes are specifically and commonly used, due to their synthetic utility [17–19], aerobic and aqueous stability [20], and tunable oxidation potential [21,22]. Thus, we believe that we could utilize these advantageous properties to synthesize an electrochemical probe purposed for the development of a point-of-care solution for the detection of potentially highly-toxic organophosphorus(III) compounds. Herein, we describe the application of a ferrocene probe to the ratiometric electrochemical detection of organophosphorus(III) compounds, including a model nerve agent precursor, using a commercial, hand-held portable potentiometer.

2. Materials and Methods

All reactions were carried out under an atmosphere of nitrogen, in oven-dried glassware, unless otherwise stated. Dichloromethane (DCM), and toluene were dried and degassed by passing through anhydrous alumina columns, using an Innovative Technology Inc. (Carouge, Switzerland) PS-400-7 solvent purification system, and were stored under an atmosphere of nitrogen prior to use. 4-Aminobenzyl alcohol was purchased from Alfa Aesar (Heysham, UK). Ferrocenecarboxaldehyde was purchased from Fluorochem (Hadfield, UK). All other chemicals were purchased from Sigma-Aldrich (Gillingham, UK). All chemicals were used as received.

Desktop electrochemical analysis was performed by applying a 20 µL sample to a screen-printed electrochemical cell equipped with carbon working and counter electrodes, and a silver (pseudo Ag/AgCl) reference electrode. The potential across the cell was powered by a Metrohm Autolab PGSTAT30 potentiostat controlled by a laptop running General Purpose Electrochemical System (GPES) software in differential pulse mode (modulation = 0.04 s, interval = 0.1 s, initial voltage = −400 mV, end voltage = 600 mV, step potential = 3 mV, modulation amplitude 49.95 mV). Post-scan, a baseline correction (moving average: peak width = 0.03) was performed. Peak integrals were obtained by using the 'peak search' function, and conversions were calculated by using the equation:

$$\text{Conversion (\%)} = (\int \text{product})/(\int \text{probe} + \int \text{product}) \times 100 \quad (1)$$

Handheld electrochemical analysis was performed by applying a 20 µL sample to a screen-printed electrochemical cell equipped with carbon working and counter electrodes, and a silver (pseudo Ag/AgCl) reference electrode. The potential across the cell was powered by a PalmSens Emstat3 Blue potentiostat controlled by a tablet, using PS Touch in differential pulse mode (equilibration time = 0 s, initial voltage = −800 mV, end voltage = 200 mV, step potential = 3 mV, pulse potential = 49.95 mV, pulse time = 0.1 s, scan rate = 0.015 V s^{-1}). Currents were obtained using the 'peak search' function, and by finding the maximum current. The currents were calibrated by using Figure S1 to obtain the concentrations, and the conversions were calculated by using the equation:

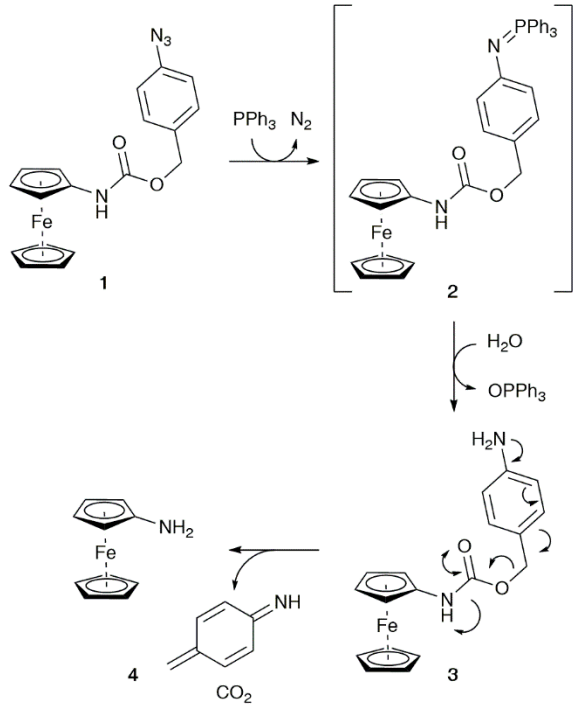
$$\text{Conversion (\%)} = ([\text{product}])/([\text{probe}] + [\text{product}]) \times 100 \quad (2)$$

3. Results

3.1. Concept

Inspired by trigger–linker–effector methodology [23,24], benzyl ferrocenylcarbamates have been effectively employed for the ratiometric electrochemical detection of both enzymes, such as β-galactosidase and alkaline phosphatase [25,26], and small molecules, such as fluoride and hydrogen peroxide [27,28]. To achieve our objective of developing a molecular probe for organophosphorus(III) species, we designed benzyl ferrocenylcarbamate **1** with a 4-azido trigger to allow for the chemoselectivity for the target to be attained through a Staudinger reaction. In the presence of the target, we proposed that the formation of iminophosphorane **2** would occur, which, under aqueous

assay conditions, would be hydrolyzed to give aniline **3**; then, a subsequent 1,6-elimination would release aminoferrocene **4** (Scheme 1). Indeed, it has since been brought to our attention that whilst this project was ongoing, the release of aminoferrocene **4** from 4-azidobenzyl ferrocenylcarbamate **1** under reductive conditions had been successfully demonstrated [29]. Due to the significantly different electronic environments surrounding the iron center, aminoferrocene **4** should have a lower oxidation potential (E_{ox}) compared with that of **1**; and, thus it should be electrochemically distinguishable.



Scheme 1. Molecular structure of probe **1**, and its proposed mechanism for the phosphine-triggered release of aminoferrocene **4**.

3.2. *Synthesis, Stability, and Electrochemical Properties of* **1**

The synthesis of **1** was successfully achieved with a 77% overall yield via a Curtius rearrangement of 4-azidobenzyl alcohol, obtained from 4-aminobenzyl alcohol through a Sandmeyer reaction, with ferrocenoyl azide, obtained from ferrocenecarboxylic acid (see Electronic Supporting Information (ESI)). Once in hand, chemodosimeter **1** remained bench-stable for several months, and more importantly, it remained stable for a month as a solution in acetonitrile at room temperature, allowing for its storage as a ready-to-use solution (Figure S2). A comparison of the electrochemical behavior of probe **1** to aminoferrocene **4** via differential pulse voltammetry (DPV) showed that the oxidation potential of probe **1** was significantly higher than the oxidation potential of aminoferrocene **4**, by 300 mV (Figure 1). Evidently, the two peaks were fully resolved allowing for conversions to be calculated from the integration of the two peaks (Figure S3), using Equation (1) (see Section 2 Materials and Methods).

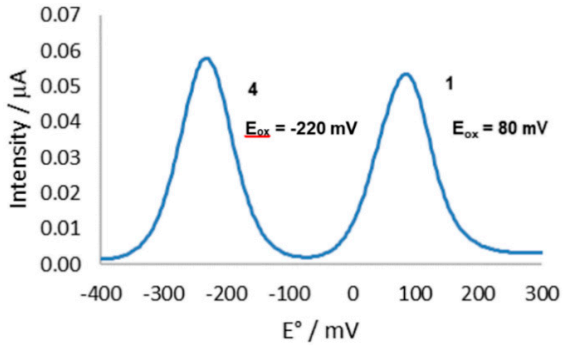
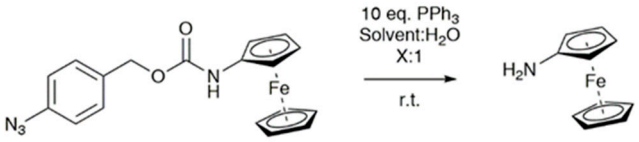


Figure 1. Differential pulse voltammogram obtained for probe **1** (0.5 mM) and aminoferrocene **4** (0.5 mM) in MeCN:Tris buffer (pH 9, 50 mM).

3.3. Sensitivity and Selectivity of **1** *towards Organophosphorus(III) Compounds*

To test the probe's response to phosphorous(III) compounds, triphenylphosphine (PPh_3) was initially selected as a model analyte, as it is an easy-to-handle solid, and it is the standard phosphine of choice in the Staudinger reaction (Table 1). Initial conditions were inspired by Staudinger ligation conditions, with 10 equivalents of triphenylphosphine in a mixed solvent system of *N*,*N*-dimethylformamide (DMF)/water. While this delivered a 37% conversion efficiency after 60 min at room temperature, changing the water-miscible co-solvent to acetonitrile (MeCN) or 1,4-dioxane improved the conversion efficiency considerably. Probe **1** was found to be insoluble in alcoholic solvents, and aminoferrocene **4** was found to be unstable in both dimethylsulfoxide (DMSO) and tetrahydrofuran (THF), thus preventing accurate electrochemical analyses, so acetonitrile was selected as the co-solvent moving forward (Table S1). Altering the solvent ratio to a 3:1 ratio of acetonitrile to water greatly improved the homogeneity of the reaction mixture, allowing for more reproducible sampling, which in turn improved the reliability of the method. Further increasing the ratio of acetonitrile to water was found to be detrimental to the reaction. Halving the equivalents of triphenylphosphine improved the accuracy, as we believed that this limited electrode fouling, though this alteration lowered the conversion down to 43%. The conversion could be increased to 80% within 20 min, and near-quantitative conversion within 60 min, by warming the assay mixture to 50 °C. At temperatures of above 50 °C, solvent loss and inaccuracies in sampling led to unreliable results.

Table 1. Optimization of the reaction assay of **1** (1 mM) with triphenylphosphine (10 mM).



1 **4**

Exp. No.	Solvent (1 M)	Solvent:Water Ratio	Conversion [1]
1	*N*,*N*-dimethylformamide (DMF)	1:1	37%
2	1,4-dioxane	1:1	78%
3	acetonitrile (MeCN)	1:1	79%
4	MeCN	3:1	79%
5 [2]	MeCN	3:1	43%
6 [3]	**MeCN**	**3:1**	**96%**

[1] Conversion determined by ratiometric electrochemical analysis. [2] Five equivalents of PPh_3. [3] 5 equivalents of PPh_3 at 50 °C.

With the optimized assay conditions in hand, the sensitivity of probe **1** was further examined (Figure 2). At superstoichiometric concentrations of triphenylphosphine (2.5 mM and 5 mM), quantitative conversion was achieved within 60 min. No background reactivity was observed in

the absence of triphenylphosphine. The negligible background rate allowed for the accurate detection, within 60 min, of low concentrations of triphenylphosphine, 50 μM, with a 3% conversion observed. This value is within an order of magnitude of the limit of detection (LOD) for the fluorescence detection of organophosphorus pesticides, using an expensive desktop fluorimeter with highly sophisticated DNA-functionalized nanoparticle detection methodology [30]. This value also corresponds to a LOD of 13 ppm, which is the value of the reported LC50 for rats [2]. At the stoichiometric equivalents of triphenylphosphine, a 40% conversion was obtained within 30 min, with no significant further increase in conversion was observed after this period. This is consistent with precedent from the literature, where it has been reported that the Staudinger reaction requires two equivalents of triphenylphosphine to release aniline [31].

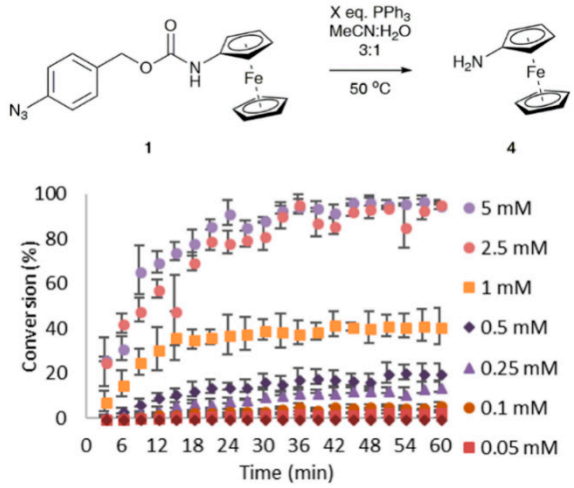


Figure 2. Conversion of probe **1** (1 mM) to aminoferrocene **4** after the addition of triphenylphosphine in MeCN:H_2O (3:1, 1M) at 50 °C. Error bars represent the standard deviation where n = 3.

To explore the selectivity of probe **1**, a selection of organophosphorus(III) compounds were screened. An analyte concentration of 1 mM was chosen, to allow for changes in the rate of reaction to be observed (Figure 3). Expectedly, electron-rich arylphosphines showed an increased reaction rate in comparison to that of triphenylphosphine (Figure S4). However, the change in electronics also corresponded to a decrease in aminoferrocene stability, which resulted in a reduced degree of reproducibility over time. Conversely, electron-deficient arylphosphines showed a significantly reduced rate of reaction in comparison to triphenylphosphine. In general, both alkylphosphines and alkylphosphites also exhibited lower rates of conversion, though pleasingly, diethylmethylphosphonite, a precursor in the synthesis of VX nerve gas, delivered a positive conversion of 8%, and it could be easily distinguished from the background. Oxygen-sensitive phosphines proved to be incompatible with the assay, due to their degradation in the assay media. Common phosphorus(V) compounds such as phosphate salts, potassium hexafluorophosphate, triphenylphosphine oxide, and triethyl phosphonoacetate yielded no conversion or breakdown of **1**, highlighting its high specificity towards phosphorus(III) species. Other soft nucleophiles such as thiols were also tested and of them, only hydrogen sulfide (H_2S) was shown to give any positive conversion with a 10% measurement after 60 min. Neither glutathione nor cysteine, both of which have also been shown to be able to reduce aryl azides [32], afforded any conversion.

The robustness of the assay was then challenged by exposing compound **1** to a series of complex samples. Specifically, crude reaction mixtures from various Suzuki cross-coupling reactions were directly injected into the assay (Figure S5). The crude reaction mixture, which contained tetrakis(triphenylphosphine)palladium(0) delivered an 87% conversion rate. This positive result was attributed to the leaching of the organophosphorus(III) ligands, as alternative Pd(0) sources, without phosphine ligands, showed minimal conversion.

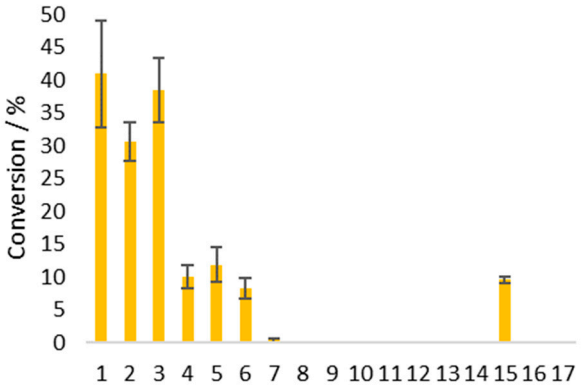


Figure 3. Conversion of probe **1** (1 mM) to aminoferrocene **4** 60 min after the addition of phosphine in MeCN:H_2O (3:1, 1M) at 50 °C. Error bars represent the standard deviation, where n = 3. (1) PPh_3; (2) P(p-tol)$_3$; (3) P(p-MeOPh)$_3$; (4) P(p-CF_3Ph)$_3$; (5) P(O^iPr)$_3$; (6) MeP(OEt)$_2$; (7) PCy_3; (8) P(OPh)$_3$; (9) PCl_2Ph; (10) K_2HPO_4; (11) KPF_6; (12) $Na_2O_7P_2$; (13) triphenylphosphine oxide; (14) triethyl phosphonoacetate; (15) hydrogen sulfide (H_2S); (16) cysteine; (17) glutathione.

3.4. Applications towards a Point-of-Use Assay with a Handheld Potentiostat

To highlight the point-of-use capability of probe **1**, the model assay was tested using a PalmSens EmStat^3 Blue handheld potentiostat (Figure S6). The lower sensitivity of the potentiostat required an increase in probe concentration to 10 mM; however, no other modifications of the reaction conditions were necessary. Due to the higher sample concentration, a corresponding reduction in sample homogeneity was observed, which resulted in a reduced reliability of the conversions, as calculated through peak integration. Therefore, in this instance, the currents were measured directly at specific oxidation potentials, and they were used to calculate the conversions via a calibration curve (Figure S7). While this does not take into account the diffusion coefficients for both probe **1** and product **4**, we believe that they are similar enough to validate this proof-of-principle experiment. To test their potential use for the detection of nerve agent precursors in the field, the modified assay was tested with the model precursor diethylmethylphosphinite (Figure 4).

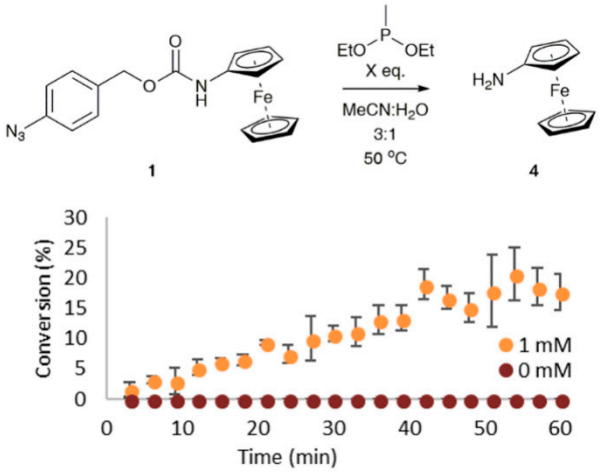


Figure 4. Conversion of probe 1 (10 mM) to aminoferrocene after the addition of diethylmethylphosphinite (X mM) in MeCN:H_2O (3:1, 1 M) at 50 °C. Error bars represent the standard deviation, where n = 3.

Interestingly, the conversion obtained was significantly higher (19 ± 3%) than that seen previously with a benchtop potentiostat (8 ± 2%). A similar increase was also observed when a comparative assay was performed while using triphenylphosphine (Figures S8–S10). We believe that the improved reactivity could be attributed to the higher concentration of the probe. More importantly, the tight error bars exhibited demonstrates the excellent reproducibility and reliability of the ratiometric detection

assay. This, coupled with the negligible background rate, highlights the potential for the direct incorporation of the assay into a future point-of-use device.

4. Conclusions

In conclusion, we have developed a ferrocene-based probe for the ratiometric electrochemical detection of phosphorus(III) compounds, utilizing the chemoselective Staudinger reaction. The probe showed excellent specificity for a range of phosphorus(III) compounds, compared against other phosphorus species, creating a highly selective and rapid detection methodology. The negligible background reactivity allowed for the reliable and reproducible detection of a model phosphine, down to 13 ppm. The detection method was successfully applied to the detection of an organophosphorus(III) nerve agent precursor, using a portable handheld potentiostat, demonstrating that the technique could be potentially applied to the rapid detection of nerve agents in the field.

Supplementary Materials: The following are available online at http://www.mdpi.com/2227-9040/7/2/19/s1, which includes the synthetic route for probe **1** (Scheme S1), and all synthetic procedures and NMR spectra for the synthesis of **1** and any intermediates synthesized during the process. Table S1: Assay optimization, Figure S1: Solution stability of probe **1**, Figure S2: Kinetic linear transformation of probe **1**, Figure S3: Voltammogram overlays, Figure S4: Extended phosphine scope, Figure S5: Complex sample testing, Figure S6: Handheld potentiostat setup, Figure S7: Calibration curve for the handheld potentiostat, Figure S8: Triphenylphosphine assay using the handheld potentiostat, Figures S9&S10: Competitive assays between desktop and handheld potentiostats.

Author Contributions: Conceptualization, S.A.S., S.G. and C.G.F.; methodology, S.G.; investigation, S.A.S.; data curation, S.A.S.; writing—original draft preparation, S.A.S.; writing—review and editing, S.G.; supervision, C.G.F.

Funding: This research received no external funding.

Acknowledgments: The authors would like to thank Professor Frank Marken for loaning the use of the PalmSens Emstat^3 Blue potentiostat, and Miss Amelia Langley for her guidance for its use.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Chaudhry, M.Q. A Review of the Mechanisms Involved in the Action of Phosphine as an Insecticide and Phosphine Resistance in Stored-Product Insects. *Pestic. Sci.* **1997**, *49*, 213–228. [CrossRef]
2. Nath, N.S.; Bhattacharya, I.; Tuck, A.G.; Schlipalius, D.I.; Ebert, P.R. Mechanisms of Phosphine Toxicity. *J. Toxicol.* **2011**, *2011*, 494168. [CrossRef] [PubMed]
3. Chaudhry, M.O.; Macnicol, A.D.; Price, N.R. Alkylphosphines as pesticidal agents. U.S. Patent 6,096,330, 1 August 2000.
4. Waritz, R.S.; Brown, R.M. Acute and Subacute Inhalation Toxicities of Phosphine, Phenylphosphine and Triphenylphosphine. *Am. Ind. Hyg. Assoc. J.* **1975**, *36*, 452–458. [CrossRef]
5. Bercu, J.P.; Galloway, S.M.; Parris, P.; Teasdale, A.; Masuda-Herrera, M.; Dobo, K.; Heard, P.; Kenyon, M.; Nicolette, J.; Vock, E.; et al. Potential impurities in drug substances: Compound-specific toxicology limits for 20 synthetic reagents and by-products, and a class-specific toxicology limit for alkyl bromides. *Regul. Toxicol. Pharmacol.* **2018**, *94*, 172–182. [CrossRef] [PubMed]
6. Brown, D.G.; Boström, J. Analysis of Past and Present Synthetic Methodologies on Medicinal Chemistry: Where Have All the New Reactions Gone? *J. Med. Chem.* **2016**, *59*, 4443–4458. [CrossRef]
7. King, A.O.; Yasuda, N. *Organometallics in Process Chemistry*; Springer: Berlin/Heidelberg, Germany, 2004; pp. 205–245, ISBN 978-3-540-01603-8.
8. Chasteen, T.G.; Fall, R.; Birks, J.W.; Martin, H.R.; Glinski, R.J. Fluorine-induced chemiluminescence detection of phosphine, alkyl phosphines and monophosphinate esters. *Chromatographia* **1991**, *31*, 342–346. [CrossRef]
9. Norman, K.N.T.; Leonard, K. Gas Chromatography−Mass Spectrometry Determination of Phosphine Residues in Stored Products and Processed Foods. *J. Agric. Food Chem.* **2000**, *48*, 4066–4070. [CrossRef] [PubMed]
10. Fahrenholtz, S.; Hühnerfuss, H.; Baur, X.; Budnik, L.T. Determination of phosphine and other fumigants in air samples by thermal desorption and 2D heart-cutting gas chromatography with synchronous SIM/Scan mass spectrometry and flame photometric detection. *J. Chromatogr. A* **2010**, *1217*, 8298–8307. [CrossRef]

11. Sun, X.; Dahlhauser, S.D.; Anslyn, E.V. New Autoinductive Cascade for the Optical Sensing of Fluoride: Application in the Detection of Phosphoryl Fluoride Nerve Agents. *J. Am. Chem. Soc.* **2017**, *139*, 4635–4638. [CrossRef]
12. Sun, X.; Boulgakov, A.A.; Smith, L.N.; Metola, P.; Marcotte, E.M.; Anslyn, E.V. Photography Coupled with Self-Propagating Chemical Cascades: Differentiation and Quantitation of G- and V-Nerve Agent Mimics via Chromaticity. *ACS Cent. Sci.* **2018**, *4*, 854–861. [CrossRef]
13. Wang, J. Electrochemical biosensors: Towards point-of-care cancer diagnostics. *Biosens. Bioelectron.* **2006**, *21*, 1887–1892. [CrossRef]
14. Ronkainen, N.J.; Halsall, H.B.; Heineman, W.R. Electrochemical biosensors. *Chem. Soc. Rev.* **2010**, *39*, 1747–1763. [CrossRef]
15. Du, Y.; Lim, B.J.; Li, B.; Jiang, Y.S.; Sessler, J.L.; Ellington, A.D. Reagentless, Ratiometric Electrochemical DNA Sensors with Improved Robustness and Reproducibility. *Anal. Chem.* **2014**, *86*, 8010–8016. [CrossRef]
16. Jin, H.; Gui, R.; Yu, J.; Lv, W.; Wang, Z. Fabrication strategies, sensing modes and analytical applications of ratiometric electrochemical biosensors. *Biosens. Bioelectron.* **2017**, *91*, 523–537. [CrossRef]
17. Beer, P.D. Redox responsive macrocyclic receptor molecules containing transition metal redox centres. *Chem. Soc. Rev.* **1989**, *18*, 409–450. [CrossRef]
18. Beer, P.D.; Gale, P.A.; Chen, G.Z. Mechanisms of electrochemical recognition of cations, anions and neutral guest species by redox-active receptor molecules. *Coord. Chem. Rev.* **1999**, *185*, 3–36. [CrossRef]
19. Kang, D.; Ricci, F.; White, R.J.; Plaxco, K.W. Survey of Redox-Active Moieties for Application in Multiplexed Electrochemical Biosensors. *Anal. Chem.* **2016**, *88*, 10452–10458. [CrossRef]
20. van Staveren, D.R.; Metzler-Nolte, N. Bioorganometallic Chemistry of Ferrocene. *Chem. Rev.* **2004**, *104*, 5931–5986. [CrossRef]
21. Marsh, B.J.; Hampton, L.; Goggins, S.; Frost, C.G. Fine-tuning of ferrocene redox potentials towards multiplex DNA detection. *New J. Chem.* **2014**, *38*, 5260–5263. [CrossRef]
22. Lim, B.J.; Hwang, I.; Ellington, A.D.; Sessler, J.L. Synthesis of Ferrocene Derivatives Allowing Linear Free Energy Studies of Redox Potentials. *Helv. Chim. Acta* **2018**, *102*, e1800186. [CrossRef]
23. Amir, R.J.; Pessah, N.; Shamis, M.; Shabat, D. "Cascade-Release Dendrimers" Liberate All End Groups upon a Single Triggering Event in the Dendritic Core. *Angew. Chem. Int. Ed.* **2003**, *42*, 4494–4499. [CrossRef]
24. Sella, E.; Shabat, D. Dendritic Chain Reaction. *J. Am. Chem. Soc.* **2009**, *131*, 9934–9936. [CrossRef]
25. Spring, S.A.; Goggins, S.; Frost, C.G. Ratiometric electrochemical detection of β-galactosidase. *Org. Biomol. Chem.* **2017**, *15*, 7122–7126. [CrossRef]
26. Goggins, S.; Naz, C.; Marsh, B.J.; Frost, C.G. Ratiometric electrochemical detection of alkaline phosphatase. *Chem. Commun.* **2015**, *51*, 561–564. [CrossRef]
27. Manibalan, K.; Mani, V.; Huang, S.T. A switchable electrochemical redox ratiometric substrate based on ferrocene for highly selective and sensitive fluoride detection. *RSC Adv.* **2016**, *6*, 71727–71732. [CrossRef]
28. Goggins, S.; Apsey, E.A.; Mahon, M.F.; Frost, C.G. Ratiometric electrochemical detection of hydrogen peroxide and glucose. *Org. Biomol. Chem.* **2017**, *15*, 2459–2466. [CrossRef]
29. Kinski, E.; Marzenell, P.; Hofer, W.; Hagen, H.; Raskatov, J.A.; Knaup, K.X.; Zolnhofer, E.M.; Meyer, K.; Mokhir, A. 4-Azidobenzyl ferrocenylcarbamate as an anticancer prodrug activated under reductive conditions. *J. Inorg. Biochem.* **2016**, *160*, 218–224. [CrossRef]
30. Dou, X.; Chu, X.; Kong, W.; Luo, J.; Yang, M. A gold-based nanobeacon probe for fluorescence sensing of organophosphorus pesticides. *Anal. Chim. Acta* **2015**, *891*, 291–297. [CrossRef]
31. Leffler, J.E.; Temple, R.D. Staudinger reaction between triarylphosphines and azides. Mechanism. *J. Am. Chem. Soc.* **1967**, *89*, 5235–5246. [CrossRef]
32. Zhang, D.; Yang, Z.; Li, H.; Pei, Z.; Sun, S.; Xu, Y. A simple excited-state intramolecular proton transfer probe based on a new strategy of thiol–azide reaction for the selective sensing of cysteine and glutathione. *Chem. Commun.* **2016**, *52*, 749–752. [CrossRef]