

Article

Syntheses and Study of a Pyrroline Nitroxide Condensed Phospholene Oxide and a Pyrroline Nitroxide Attached Diphenylphosphine

Mostafa Isbera ¹, Balázs Bognár ¹ , Ferenc Gallyas ^{2,3,4}, Attila Bényei ⁵ , József Jekő ⁶ and Tamás Kálai ^{1,4,*}

¹ Faculty of Pharmacy, Institute of Organic and Medicinal Chemistry, University of Pécs, Szigeti st. 12, H-7624 Pécs, Hungary; mostafaisbera@gmail.com (M.I.); balazs.bognar@aok.pte.hu (B.B.)

² Department of Biochemistry and Medical Chemistry, University of Pécs Medical School, H-7624 Pécs, Hungary; ferenc.gallyas@aok.pte.hu

³ HAS-UP Nuclear-Mitochondrial Interactions Research Group, H-1245 Budapest, Hungary

⁴ János Szentágothai Research Center, University of Pécs, Ifjúság 20, H-7624 Pécs, Hungary

⁵ Department of Pharmaceutical Chemistry, University of Debrecen, Egyetem tér 1, H-4032 Debrecen, Hungary; benyei.attila@science.unideb.hu

⁶ Department of Chemistry, University of Nyíregyháza, Sóstói st. 31/B, H-4440 Nyíregyháza, Hungary; jjozsi@gmail.com

* Correspondence: tamas.kalai@aok.pte.hu; Tel.: +36-72-536-221



Citation: Isbera, M.; Bognár, B.; Gallyas, F.; Bényei, A.; Jekő, J.; Kálai, T. Syntheses and Study of a Pyrroline Nitroxide Condensed Phospholene Oxide and a Pyrroline Nitroxide Attached Diphenylphosphine.

Molecules **2021**, *26*, 4366.
<https://doi.org/10.3390/molecules26144366>

Academic Editor: M. Amparo F. Faustino

Received: 21 May 2021
Accepted: 14 July 2021
Published: 19 July 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: The reaction of a diene nitroxide precursor with dichlorophenylphosphine in a McCormac procedure afforded 1,1,3,3-tetramethyl-5-phenyl-1,2,3,4,5,6-hexahydrophospholo[3,4-c]pyrrole-5-oxide-2-oxyl. Lithiation of the protected 3-iodo-pyrroline nitroxide followed by treatment with chlorodiphenylphosphine after deprotection afforded (1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-yl)diphenylphosphine oxide, and after reduction, (1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-yl)diphenylphosphine was realized, which was also supported by X-ray single crystal diffraction measurements. This pyrroline diphenylphosphine derivative was converted to hexadecylphosphonium salt, which is an analogue of antineoplastic agent, MITO-CP.

Keywords: lithiation; McCormac reaction; nitroxides; protecting groups; reduction

1. Introduction

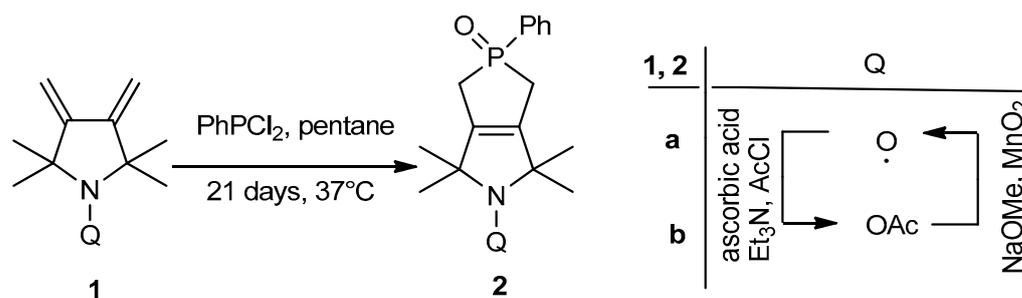
Heterocyclic nitroxide derivatives of piperidine pyrrolidine, pyrroline and imidazole have found various applications [1,2]. Stable nitroxide (aminoxyl) free radicals possess unique properties and reactivity, and despite decades of experience working with them, accessing new scaffolds without affecting the free valence is a challenge for every synthetic chemist active in this field [3]. Sometimes nitroxide moieties require temporary protection [4,5] to avoid irreversible destruction during various chemical transformations. Nitroxides have a wide range of applications; they are used as co-oxidants in organic chemistry, building blocks for magnetic materials, superoxide dismutase mimics, antiproliferative compounds, mediators of polymerization, redox active materials in batteries, magnetic resonance imaging (MRI), and electron paramagnetic resonance imaging (EPRI) contrast agents [1,2]. These various applications require numerous paramagnetic scaffolds adjusted to different utilizations. Our idea is to combine various phosphorus-containing functional groups with nitroxides, which may offer fascinating possibilities for synthetic, structural and biological studies and the utilization of these new compounds [6,7]. In continuation of our interest in the synthesis of nitroxide-based phosphorus compounds, we have promoted the synthesis of phosphorus-containing heterocycles condensed with pyrroline nitroxide and pyrroline nitroxide-diphenylphosphine and pyrroline nitroxide-diphenylphosphine oxide compounds. Although similar nitroxides have been previously reported with nitronyl nitroxides [8] and a phosphorus-containing heterocycle [9], our

approach described in the present work might open a new route for synthesizing such novel types of paramagnetic phosphorus-containing compounds.

2. Results and Discussions

2.1. Synthesis of Paramagnetic Phospholene Oxide

A standard procedure to form phospholene oxide is McCormac cycloaddition attempted from compound **1a** [10] and dichlorophenylphosphine [11,12]. However, this addition did not give the expected, isolable product. Proposing the disruption of the nitroxide under the reaction conditions applied, we protected nitroxide as an *O*-acetyl derivative **1b** [4]. Treatment of compound **1b** with dichlorophenylphosphine in a three-week-long reaction time in pentane at 37 °C enabled us to obtain compound **2b** after hydrolysis with a modest 36% yield. Deprotection of the *O*-acetyl group by a catalytic amount of NaOMe, followed by oxidation of the *N*-hydroxylamine with MnO₂, offered 1,1,3,3-tetramethyl-5-phenyl-1,2,3,4,5,6-hexahydrophospholo[3,4-*c*]pyrrole-5-oxide-2-oxyl **2a** as the first pyrroline nitroxide condensed phospholene oxide (Scheme 1). Compound **2a** was reduced to its hydroxylamine derivative in situ in the NMR tube (see Section 3.1.) and in the resulted ³¹P-NMR we found a single peak at 61.8 ppm and methylene protons as multiplets 2.68–2.73 and 2.85–2.29 ppm with 2H–2H integrals, suggesting that compound **2a** contains an endocyclic double bond.



Scheme 1. Synthesis of a paramagnetic phospholene oxide via the McCormack reaction.

2.2. Synthesis of Pyrroline Nitroxide Diphenylphosphine and Its Phosphonium Salt

As triphenylphosphine is an essential building block of mitochondria-targeted antioxidants and neoplastic agents [13,14], e.g., lipophilic triphenylphosphonium cations, we aimed to synthesize the paramagnetic analog of triphenylphosphine. We intended to determine whether or not we can synthesize different types of nitroxide-containing mitochondrially targeted molecules compared to MITO-CP (Figure 1) [15]. In our case, nitroxide would function as a superoxide dismutase (SOD) mimic at the cationic “warhead”.

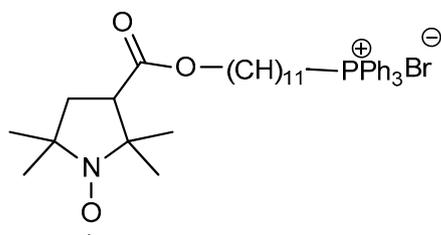
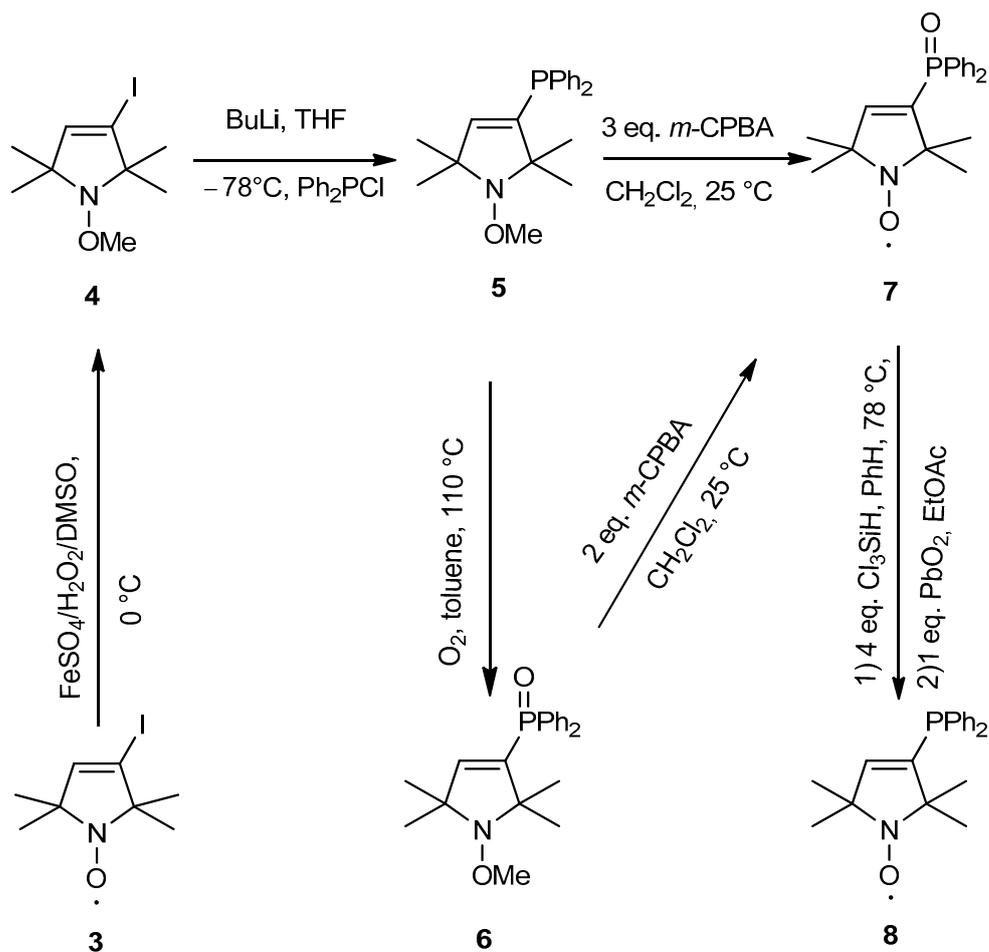


Figure 1. Structure of MITO-CP.

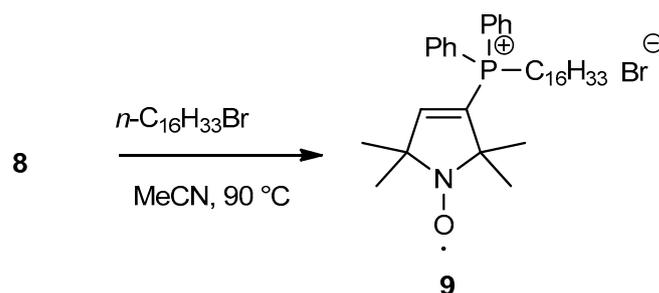
Compound **3** [16] was converted to the corresponding **4** *O*-methyl derivative in a coupled Fenton reaction, which generates methyl radicals. This protected pyrroline nitroxide **4** was treated with 1.1 eq. BuLi followed by the addition of diphenylchlorophosphine to furnish compound **5**. Phosphine **5** proved to be rather stable during the flash chromatography purification process and could be stored for weeks under an Ar atmosphere

at $-18\text{ }^{\circ}\text{C}$ without oxidation (e.g., appearance of compound **6**). However, refluxing in toluene in air oxidized it to phosphine **6** oxides. Treatment of compounds **5** and **6** with *meta*-chloroperbenzoic acid (*m*-CPBA) to remove the protecting methyl group from the oxygen atom furnished paramagnetic phosphine oxide **7**. Compound **7** could be reduced with paramagnetic pyrroline nitroxide diphenylphosphine by heating it with 4 eq. trichlorosilane in toluene at $80\text{ }^{\circ}\text{C}$ to reduce the phosphinoyl function [17,18] to phosphine and reduce nitroxide to hydroxylamine. The latter could be selectively oxidized to nitroxide **8** by PbO_2 (Scheme 2) without oxidation of phosphorus.



Scheme 2. Synthesis of pyrroline nitroxide diphenylphosphine and pyrroline nitroxide diphenylphosphin oxide.

Compound **8** was heated with hexadecylbromide for 5 days in acetonitrile at $90\text{ }^{\circ}\text{C}$ in a closed vial to afford compound **9** in a low 5% yield in a sluggish reaction, presumably because of sterical hindrance due to the pyrroline nitroxide ring and because of side-reactions (Scheme 3).



Scheme 3. Synthesis of a paramagnetic phosphonium salt.

2.3. X-ray Crystallographic Study of Pyrroline Nitroxide-Diphenylphosphine

X-ray-quality crystals of **8** were grown by slow crystallization from pentane/Et₂O (2:1) solution by spontaneous evaporation of the solvent. A suitable crystal was fixed under a microscope onto a Mitegen loop using high-density oil. Diffraction intensity data were collected at 200 K using a Bruker-D8 Venture diffractometer (Bruker AXS GmbH, Karlsruhe, Germany) equipped with INCOATEC I μ S 3.0 (Incoatec GmbH, Geesthacht, Germany) dual (Cu and Mo) sealed tube micro sources and a Photon II Charge-Integrating Pixel Array detector (Bruker AXS GmbH, Karlsruhe, Germany) using Mo K α ($\lambda = 0.71073$ Å) radiation. High multiplicity data collection and integration were performed using APEX3 (version 2017.3-0, Bruker AXS Inc., 2017, Madison, WI, USA) software. Data reduction and multiscan absorption correction were performed using SAINT (version 8.38A, Bruker AXS Inc., 2017, Madison, WI, USA). The structure was solved using direct methods and refined on F² using the SHELXL program [19] incorporated into the APEX3 suite. Refinement was performed anisotropically for all nonhydrogen atoms. Hydrogen atoms were placed into geometric positions. The CIF file was manually edited using PubCIF software [20], while graphics were prepared using the Mercury program [21]. The results for the X-ray diffraction structure determinations were very good according to the CheckCIF functionality of PLATON software (Utrecht University, Utrecht, The Netherlands) [22], and structural parameters such as bond length and angle data were in the expected range (for selected data, see the caption for Figure 2). The crystallographic and refinement details are given in Table 1. CCDC contains the supplementary crystallographic data for **8** with deposition number 2082286. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif accessed on 21 May 2021.

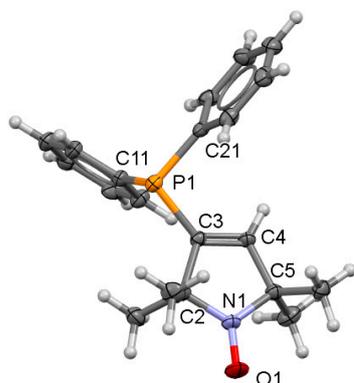


Figure 2. Ortep style view of **8** with a partial numbering scheme showing thermal displacement ellipsoids drawn at the 50% probability level. Key bond lengths [Å] and angles [°] are P1-C3 1.827(2), P1-C21 1.835(2), P1-C11 1.827(2), N1-O1 1.271(3), C3-C4 1.322(3), N1-C2 1.480(3), N1-C5 1.476(3); O1-N1-C5 122.2(2), C5-N1-C2 114.94(18), C4-C3-P1 129.02(18), C2-C3-P1 119.46(17).

Table 1. Crystal data for compound **8**.

Chemical Formula	C₂₀H₂₃NO
M _r	324.36
Crystal system, space group	Monoclinic, P2 ₁
Temperature (K)	200
<i>a</i> , <i>b</i> , <i>c</i> (Å)	8.3032 (3), 12.1786 (4), 9.3477 (3)
β (°)	108.792 (2)
<i>V</i> (Å ³)	894.86 (5)
<i>Z</i>	2
Radiation type	Mo Kα
μ (mm ⁻¹)	0.16
Crystal size (mm)	0.45 × 0.44 × 0.28
Data collection	
Diffractometer	Bruker D8 VENTURE
Absorption correction	Multiscan SADABS2016/2-Bruker AXS area detector scaling and absorption correction
<i>T</i> _{min} , <i>T</i> _{max}	0.85, 0.96
No. of measured, independent, and observed (<i>I</i> > 2σ(<i>I</i>)) reflections	13,054, 3528, 3427
<i>R</i> _{int}	0.037
(sin θ/λ) _{max} (Å ⁻¹)	0.619
Refinement	
<i>R</i> (<i>F</i> ² > 2σ(<i>F</i> ²)), <i>wR</i> (<i>F</i> ²), <i>S</i>	0.031, 0.096, 1.21
No. of reflections	3528
No. of parameters	213
No. of restraints	1
H-atom treatment	H-atom parameters constrained
Δ _{max} , Δ _{min} (e Å ⁻³)	0.51, −0.50
Absolute structure	Flack <i>x</i> determined using 1581 quotients [(<i>I</i> ⁺) − (<i>I</i> [−])]/[(<i>I</i> ⁺) + (<i>I</i> [−])] (Parsons, Flack and Wagner, Acta Cryst. B69 (2013) 249–259).
Absolute structure parameter	−0.05 (3)

The molecular structure of **8** as the targeted nitroxide derivative of the diphenylphosphinopyrrole derivative is fully supported by the X-ray diffraction study. Both the N-O distance and the double bond between C3 and C4 (see Figure 2) were proven. A search of the Cambridge Structural Database (version 5.41 Updates March 2020) [23] revealed 73 hits for similar 2,2,5,5 tetramethyl pyrrole nitroxide compounds, with an average N-O distance of 1.278(33) Å. We observed a similar value of 1.271(3) Å. However, no phosphorous derivative at C3 or C4 could be found, showing the uniqueness of our compound. The compound crystallized in the monoclinic space group P2₁, is chiral. Moreover, the Flack parameter is very close to 0 (Table 1) indicating that we have a chiral lattice for our achiral molecule. The packing diagram shows a very small portion of the unit cell as a void (Figure 3).

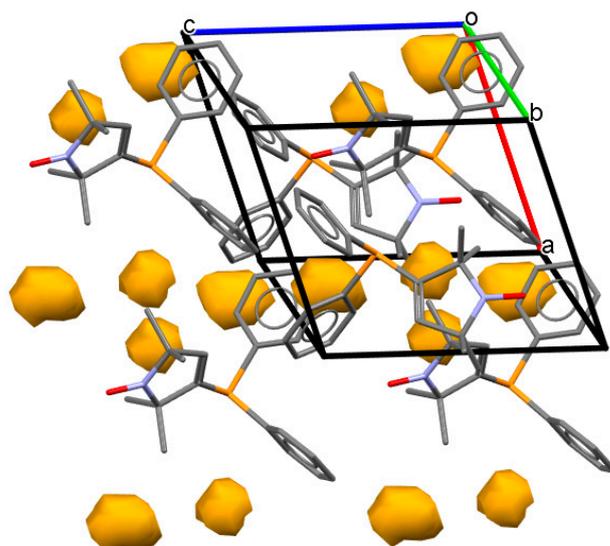


Figure 3. Packing diagram of **8**. Hydrogen atoms are omitted for clarity.

3. Materials and Methods

3.1. General Methods and Reagents

The mass spectra were recorded with a GCMS-2020 (Shimadzu, Tokyo, Japan) operated in EI mode (70 eV) and a ThermoScientific Q-Extractive HPLC/MS/MS with ESI(+) ionization (Thermo Scientific, Waltham, MA, USA). Elemental analyses were carried out with a Fisons EA 1110 CHNS elemental analyzer (Fisons Instruments, Milan, Italy). The melting points were determined with a Boetius micromelting point apparatus (Franz Küstner Nachf. K. G., Dresden, Germany). ^1H NMR spectra were recorded with a Bruker Avance 3 Ascend 500 system (Bruker BioSpin Corp., Karlsruhe, Germany) operated at 500 MHz, and ^{13}C NMR spectra were obtained at 125 MHz and ^{31}P NMR 202 MHz in CDCl_3 or $\text{DMSO}-d_6$ at 298 K. The “in situ” reduction of nitroxides was achieved by the addition of five equivalents of hydrazobenzene (DPPH/radical). The EPR spectra were recorded using a MiniScope MS 200 (Magnettech GMBH, Berlin, Germany) in CHCl_3 solution, and the sample concentrations were 1.0×10^{-4} M. All monoradicals gave a triplet line at $a_{\text{N}} = 14.4$ G. IR spectra were obtained with a Bruker Alpha FT-IR instrument (Bruker Optics, Ettlingen, Germany) with ATR support on a diamond plate. All spectra are shown in the Supplementary Material. Flash column chromatography was performed using a Merck Kieselgel 60 (0.040–0.063 mm). Qualitative TLC was carried out using commercially available plates (20 × 20 × 0.02 cm) coated with Merck Kieselgel (Darmstadt, Germany) GF₂₅₄. Compounds **1a** [9], **3** [15] were synthesized as previously reported. All the other reagents were purchased from Sigma Aldrich (St. Louis, MO, USA), Molar Chemicals (Halásztelek, Hungary).

3.2. Synthesis of 2,2,5,5-Tetramethyl-3,4-dimethylenepyrrolidin-1-yl Acetate (**1b**)

A solution of ascorbic acid (8.80 g, 50.0 mmol) in H_2O (10 mL) was added to a solution of radical **1a** (1.66 g, 10.0 mmol) in dioxane (30 mL), and the mixture was stirred at 40 °C for 15 min under N_2 . The pale-yellow solution was extracted with CHCl_3 (2 × 20 mL) and dried on MgSO_4 under N_2 . Acetyl chloride (860 mg, 10.0 mmol) was added at 0 °C, followed by the slow addition of Et_3N (1.10 g, 11.0 mmol) at this temperature. Stirring was continued for 1 h at room temperature, and after adding ethanol (1 mL), the reaction mixture was filtered, and the filtrate was evaporated to dryness. The residue was partitioned between brine (15 mL) and EtOAc (20 mL). The organic phase was separated, and the aqueous phase was washed with EtOAc (2 × 10 mL). The combined organic phase was dried (MgSO_4), filtered, and evaporated, and after flash chromatography purification (hexane- Et_2O , 6:1), we obtained compound **1b** (1.79 g, 86%) as a white solid, mp 35–37 °C; TLC

(hexane/Et₂O, 5:1): R_f = 0.43, IR: 2974, 2933, 1766, 1618 cm⁻¹. ¹H NMR (CDCl₃) δ: 1.30 (s, 12H, 2 × C(CH₃)₂), 2.16 (s, 3H, CH₃CO), 4.89 (s, 2H, CH₂), 5.44 (s, 2H, CH₂), ¹³C NMR (CDCl₃) δ: 19.1 (COCH₃), 29.6 (4C, C(CH₃)₂), 66.4 (2C, C(CH₃)₂), 104.2 (2C, H₂C=), 151.1 (2C, C=), 171.2 (1C, C=O). MS (EI): *m/z* (%): 209 (M⁺, 1), 194 (11), 167 (14), 152 (100), 120 (16), 43 (9). Anal. calcd. for C₁₂H₁₉NO₂: C, 68.87; H, 9.15; N, 6.69%; found: C, 68.75; H, 8.95; N, 6.59%.

3.3. Synthesis of 1,1,3,3-Tetramethyl-5-oxido-5-phenyl-5,6-dihydrophospholo [3,4-*c*]pyrrol-2(1H,3H,4H)-yl Acetate (2b)

Compound **1b** (1.88 g, 9.0 mmol), Cu(II) stearate (100 mg, 0.15 mmol as polymerization inhibitor), and pentane (10 mL) were added together with dichlorophenyl phosphine (3.22 g, 18.0 mmol) dropwise into a pressure-proof tube equipped with a magnetic stirrer, with stirring at 0 °C. After addition, the tube was capped and stirred at room temperature for 4 days followed by stirring for 17 days at 37 °C. After cooling, the solution was poured into a 250 mL beaker containing a mixture of ice (40 g) and sat. NaHCO₃ solution (40 mL). The tube was rinsed with NaHCO₃ solution (10 mL), CHCl₃ (10 mL) was poured into the beaker, CHCl₃ (80 mL) was added, and the two-phase system was stirred for 15 min.

After separation of the organic phase, the aqueous phase was washed with CHCl₃ (2 × 10 mL), and the combined organic phases were dried (MgSO₄), filtered, and evaporated. The residue was purified by flash column chromatography (hexane/EtOAc 2:1 followed by CHCl₃/Et₂O 1:4) to give compound **2b** as a white solid (1.07 g, 36%); mp 150–152 °C; TLC (CHCl₃/MeOH, 58:2): R_f = 0.41, IR: 2974, 2927, 1767 cm⁻¹. ³¹P NMR (CDCl₃) δ: 61.7; ¹H NMR (CDCl₃) δ: 1.29 (s, 12H, 2 × C(CH₃)₂), 2.18 (s, 3H, CH₃CO), 2.68–2.73 (m, 2H, CH₂), 2.79–2.85 (m, 2H, CH₂), 7.50–7.74 (m, 5H, ArH). ¹³C NMR (CDCl₃) δ: 19.2. (COCH₃), 21.7 (2C, C(CH₃)₂), 27.1 (2C, C(CH₃)₂), 31.2 (*d*, *J* = 65.5 Hz, 2C, CH₂), 128.9 (*d*, 2C, ArC *J* = 11 Hz), 129.2. (*d*, 2C, *J* = 10.0 Hz, ArC) 132.3 (1C, ArC), 134.4 (*d*, 1C, ArC, *J* = 94 Hz), 139.4 (2C, C=, *J* = 11 Hz), 170.9 (1C, C=O); MS (EI): *m/z* (%): 333 (M⁺, 1), 318 (19), 291 (54), 276 (100), 259 (38). Anal. calcd. for C₁₈H₂₄NO₃P: C, 64.85; H, 7.26; N, 4.20 %; found: C, 64.65; H, 7.15; N, 4.17%.

3.4. Synthesis of 1,1,3,3-Tetramethyl-5-phenyl-5-oxido-1,2,3,4,5,6-hexahydrophospholo [3,4-*c*]pyrrole-2-yloxyl Radical (2a)

Freshly prepared NaOMe (from 12 mg, 0.52 mmol Na dissolved in 5 mL MeOH) was added to a solution of compound **2b** (999 mg, 3.0 mmol) in MeOH (20 mL), and the mixture was allowed to remain for 2 h at 25 °C. The solvent was evaporated off, the residue was dissolved in sat. aq. NH₄Cl solution (10 mL) and extracted with CHCl₃ (2 × 20 mL). The combined organic phase was dried (MgSO₄), MnO₂ (86 mg, 1.0 mmol) was added, and O₂ was bubbled through for 10 min. After filtration, the reaction mixture was evaporated and purified by flash column chromatography (CHCl₃/Et₂O, 2:1) to furnish the title compound as a yellow solid (826 mg, 95%); mp 194–197 °C; TLC (CHCl₃/MeOH, 58:2): R_f = 0.37, IR: 2974, 2827 cm⁻¹. ³¹P NMR (CDCl₃ + (PhNH)₂) δ: 61.8; ¹H NMR (CDCl₃ + (PhNH)₂) δ: 1.34 (s, 12H, 2 × C(CH₃)₂), 2.70 (*dd*, 2H, CH₂ *J*¹ = 7.0 Hz, *J*² = 7.0 Hz), 2.88 (t, 2H, CH₂ *J* = 16 Hz), aromatic protons overlap with (PhNH)₂ protons. ¹³C NMR (CDCl₃) δ: 23.7 (2C, C(CH₃)₂), 24.5 (2C, C(CH₃)₂), 31.2 (*d*, *J* = 67 Hz, 2C, CH₂), 68.4 (*d*, 2C, C(CH₃)₂, *J* = 9 Hz), 128.9 (*d*, 2C, ArC, *J* = 11 Hz), 129.3 (2C, ArC), 132.2 (1C, ArC), 134.3 (*d*, 1C, ArC, *J* = 88 Hz), 139.8 (2C, C=, *J* = 11 Hz), MS (EI): *m/z* (%): 290 (M⁺, 13), 275 (31), 260 (73), 245 (100). Anal. calcd. for C₁₆H₂₁NO₂P: C, 66.19; H, 7.29; N, 4.82%; found: C, 66.05; H, 7.15; N, 4.84%.

3.5. Synthesis of 3-Iodo-1-methoxy-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrole (4)

First, 30% aq. H₂O₂ (5 mL) was added dropwise to a stirred solution of compound **3** (2.66 g, 10.0 mmol) and FeSO₄·7H₂O (6.9 g, 25.0 mmol) in DMSO (30 mL) at 0 °C, over 2 h. The reaction was monitored by TLC. Upon consumption of the starting material, the reaction mixture was diluted with water (50 mL) and 10% aq. Na₂SO₃ (25 mL). The aqueous solution was extracted with Et₂O (3 × 30 mL). The combined organic phases were dried (MgSO₄), filtered, and evaporated, and the crude product was purified by flash column chromatography (hexane/Et₂O, 2:1) to give a colorless oil (2.24 g, 80%); TLC (hexane/Et₂O,

58:2); $R_f = 0.52$. IR: 2973, 2894, 1610 cm^{-1} . ^1H NMR (CDCl_3) δ : 1.24 (s, 12H, $2 \times \text{C}(\text{CH}_3)_2$), 3.67 (s, 3H, CH_3CO), 5.94 (s, 1H, $\text{HC}=\text{C}$). ^{13}C NMR (CDCl_3) δ : 22.2 (2C, $\text{C}(\text{CH}_3)_2$), 29.5 (2C, $\text{C}(\text{CH}_3)_2$), 64.9 (1C, OCH_3), 70.7 (1C, $\text{C}(\text{CH}_3)_2$), 72.9 (1C, $\text{C}(\text{CH}_3)_2$), 101.6 (1C, $\text{IC}=\text{C}$), 142.8 (1C, $\text{HC}=\text{C}$). MS (EI): m/z (%): 281 (M^+ , 5), 266 (97), 139 (100), 108 (33), 83 (43). Anal. calcd. for $\text{C}_9\text{H}_{16}\text{INO}$: C, 38.45; H, 5.74; N, 4.98 %; found: C, 38.36; H, 5.65; N, 4.99%.

3.6. Synthesis of 3-(Diphenylphosphino)-1-methoxy-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrole (5)

n-BuLi solution in hexane (2.8 mL, 7.0 mmol, 2.5 M) diluted with anhydr. THF (10 mL) was added dropwise to a stirred solution of compound 4 (1.97 g, 7.0 mmol) in anhydr. THF (10 mL) at -78°C under N_2 . After the addition was completed, the mixture was continuously stirred for 1 h at -78°C . Then, a solution of diphenylchlorophosphine (1.55 g, 7.0 mmol) in anhydr. THF (10 mL) was added dropwise. After stirring at this temperature for 30 min, the reaction mixture was allowed to warm to r.t. with continuous stirring for 2 h. A sat. aq. NH_4Cl solution (5 mL) was added, the mixture was extracted with EtOAc (2×20 mL), the combined organic phase was dried (MgSO_4), filtered and evaporated, and the crude product was purified by flash column chromatography (hexane/ Et_2O , 4:1) to give a white powder (1.6 g, 67%); mp $95\text{--}97^\circ\text{C}$ TLC (hexane/ Et_2O , 58:2); $R_f = 0.31$. IR: 3056, 2971, 1620, 1572 cm^{-1} . ^{31}P NMR (CDCl_3) δ : -27.0 ; ^1H NMR (CDCl_3) δ : 1.26 (s, 12H, $2 \times \text{C}(\text{CH}_3)_2$), 3.70 (s, 3H, OCH_3), 5.28 (s, 1H, $\text{HC}=\text{C}$), 7.37 (bs, 10H, ArH). ^{13}C NMR (CDCl_3) δ : 23.0 (2C, $\text{C}(\text{CH}_3)_2$), 29.6 (2C, $\text{C}(\text{CH}_3)_2$), 65.0 (1C, OCH_3), 68.5 (1C, $\text{C}(\text{CH}_3)_2$), 73.1 (*d*, 1C, $\text{C}(\text{CH}_3)_2$, $J = 24$ Hz), 128.35 (*d*, 4C, ArC, $J = 7$ Hz), 128.7 (2C, ArC), 133.9 (*d*, 4C, ArC, $J = 20$ Hz), 135.8 (*d*, 2C, ArC, $J = 16$ Hz), 143.2 (*d*, 1C, $=\text{CP}$, $J = 18$ Hz), 143.5 (*d*, 1C, $\text{HC}=\text{C}$, $J = 2.6$ Hz), MS (EI): m/z (%): 339 (M^+ , 5), 324 (100), 293 (78), 201 (12), 185 (45), 108 (44). Anal. calcd. for $\text{C}_{21}\text{H}_{26}\text{NOP}$: C, 74.31; H, 7.72; N, 4.13%; found: C, 74.21; H, 7.66; N, 4.11%.

3.7. Synthesis of 1-Methoxy-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-yl)diphenylphosphine Oxide (6)

A solution of compound 5 (679 mg, 2.0 mmol) in toluene (15 mL) was heated at the reflux temperature for 12 h. The solvent was evaporated, and the crude material was subjected to flash column chromatography (hexane/EtOAc, 2:1) to give a yellow powder (355 mg, 50%); mp $128\text{--}130^\circ\text{C}$; TLC ($\text{CHCl}_3/\text{Et}_2\text{O}$, 2:1); $R_f = 0.46$. IR: 2973, 2674, 1606 cm^{-1} . ^{31}P NMR (CDCl_3) δ : 23.5; ^1H NMR (CDCl_3) δ : 1.28 (s, 6H, $\text{C}(\text{CH}_3)_2$), 1.36 (s, 6H, $\text{C}(\text{CH}_3)_2$), 3.65 (s, 3H, OCH_3), 5.68 (*d*, 1H, $\text{HC}=\text{C}$, $J = 11.5$ Hz), 7.48–7.72 (m, 10H, ArH). ^{13}C NMR (CDCl_3) δ : 22.7 (2C, $\text{C}(\text{CH}_3)_2$), 29.7 (2C, $\text{C}(\text{CH}_3)_2$), 65.1 (1C, OCH_3), 68.8 (*d*, 1C, $\text{C}(\text{CH}_3)_2$, $J = 13$ Hz), 73.2 (*d*, 1C, $\text{C}(\text{CH}_3)_2$, $J = 11$ Hz), 128.4 (*d*, 4C, ArC, $J = 12$ Hz), 131.75 (2C, ArC), 131.79 (*d*, 4C, ArC, $J = 4$ Hz), 132.3 (*d*, 2C, ArC, $J = 124$ Hz), 138.6 (*d*, 1C, $\text{PC}=\text{C}$, $J = 101$ Hz), 150.1 (*d*, 1C, $=\text{CH}$, $J = 8$ Hz). MS (EI): m/z (%): 355 (M^+ , 2), 340 (90), 310 (10), 308 (45), 201 (100), 108 (25). Anal. calcd. for $\text{C}_{21}\text{H}_{26}\text{NO}_2\text{P}$: C, 70.97; H, 7.37; N, 3.94%; found: C, 71.07; H, 7.41; N, 3.87%.

3.8. Synthesis of 3-(Diphenylphosphinoxido)-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-1-yloxy Radical (7)

First, 3-chloroperbenzoic acid (3.0 eq. for compound 5, 2.0 eq. for compound 6) was added in 2–3 portions at 0°C to a stirred solution of compound 5 or 6 (2.0 mmol) in anhydr. CH_2Cl_2 (5 mL) for 10 min. The solution was stirred for an additional 30 min at ambient temperature. Then, the solution was washed with 10% aq. Na_2CO_3 solution (2×10 mL), and the organic phase was separated, dried (MgSO_4), filtered, and evaporated. The residue was subjected to flash column chromatography (hexan-EtOAc, 1:9) to give a yellow powder (612 mg, 90%); mp $135\text{--}137^\circ\text{C}$; TLC ($\text{CHCl}_3/\text{MeOH}$, 58:2); $R_f = 0.56$. IR: 2973, 2925, 1596 cm^{-1} . ^{31}P NMR ($\text{CDCl}_3+(\text{PhNH})_2$) δ : 23.4; ^1H NMR ($\text{CDCl}_3+(\text{PhNH})_2$) δ : 1.32 (s, 6H, $\text{C}(\text{CH}_3)_2$), 1.42 (s, 6H, $\text{C}(\text{CH}_3)_2$), 5.82 (*d*, 1H, $\text{HC}=\text{C}$, $J = 11.5$ Hz), aromatic protons overlap with $(\text{PhNH})_2$ signals. ^{13}C NMR (CDCl_3) δ : 25.1 (2C, $\text{C}(\text{CH}_3)_2$), 25.8 (2C, $\text{C}(\text{CH}_3)_2$), 69.1 (*d*, 1C, $\text{C}(\text{CH}_3)_2$, $J = 12$ Hz), 73.4 (*d*, 1C, $\text{C}(\text{CH}_3)_2$, $J = 11$ Hz), 128.5 (*d*, 4C, ArC, $J = 12$ Hz), 131.8 (*d*, 4C, ArC, $J = 10$ Hz), 131.90 (s, 2C, ArC), 132.6 (*d*, 2C, ArC, $J = 4$ Hz), 138.9 (*d*, 1C, $\text{PC}=\text{C}$, $J = 101$ Hz), 149.8 (*d*, 1C, $=\text{CH}$, $J = 8$ Hz). MS (EI): m/z (%): 340 (M^+ , 92),

310 (10), 308 (45), 201 (100), 108 (20). Anal. calcd. for $C_{20}H_{23}NO_2P$: C, 70.57; H, 6.81; N, 4.12%; found: C, 70.71; H, 6.77; N, 4.01%.

3.9. Synthesis of 3-(Diphenylphosphino)-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-1-ylloxyl Radical (8)

Trichlorosilane (0.8 mL, 4 eq., 8 mmol) was added to a stirred solution of compound 7 (680 mg, 2.0 mmol) in anhydr. toluene (10 mL), at 0 °C under N_2 . The resulting mixture was stirred at 80 °C for 4 h under N_2 , cooled to room temperature and then poured into a 250 mL beaker containing ice (40 g) and 5% aq. NaOH solution (10 mL). The organic phase was extracted with EtOAc (2 × 15 mL), dried ($MgSO_4$), oxidized by adding PbO_2 (478 mg, 2.0 mmol), filtered, and then evaporated. The residue was purified by flash column chromatography (hexane/ Et_2O) to give a yellow powder (421 mg, 65%); mp 120–122 °C; TLC (hexane/ Et_2O , 2:1): R_f = 0.42. IR: 3054, 2974, 2855, 1598, 1582 cm^{-1} . ^{31}P NMR ($CDCl_3+(PhNH)_2$) δ : -26.6; 1H NMR ($CDCl_3+(PhNH)_2$) δ : 1.32 (s, 6H, $C(CH_3)_2$), 1.34 (s, 6H, $C(CH_3)_2$), 5.45 (d, 1H, HC=, J = 4 Hz), aromatic protons overlap with $(PhNH)_2$ signals. ^{13}C NMR ($CDCl_3$) δ : 25.1 (2C, $C(CH_3)_2$), 25.8 (2C, $C(CH_3)_2$), 69.1 (d, 1C, $C(CH_3)_2$, J = 12 Hz) 73.4 (d, 1C, $C(CH_3)_2$, J = 11 Hz), 128.4 (d, 4C, ArC, J = 7 Hz), 128.9 (2C, ArC), 131.8 (d, 2C, ArC, J = 10 Hz), 134.0 (d, 4C, ArC, J = 20 Hz), 135.7 (d, 1C, PC=, J = 8 Hz), 143.3 (d, 1C, =CH, J = 2 Hz). MS (EI): m/z (%): 324 (M^+ , 17), 294 (99), 279 (100), 183 (62), 108 (84). Anal. calcd. for $C_{20}H_{23}NOP$: C, 74.05; H, 7.15; N, 4.32%; found: C, 74.21; H, 6.94; N, 4.46%.

3.10. Synthesis of Hexadecyl (1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-yl) diphenylphosphonium Bromide Radical (9)

A mixture of compound 8 (324 mg, 1.0 mmol) and hexadecylbromide (305 mg, 1.0 mmol) in acetonitrile (10 mL) in a pressure-proof closed vial was stirred and heated at 90 °C for 5 days. After cooling to room temperature, the solvent was evaporated, and the crude material was purified by flash column chromatography ($CHCl_3/MeOH$, 9:1) to furnish the title compound as a beige solid (31 mg, 5%); mp 118–120 °C; TLC ($CHCl_3/MeOH$, 9:1): R_f = 0.40, IR: 2923, 2852, 1598 cm^{-1} . 1H and ^{13}C NMR data were not obtained because of the low solubility of the title compound; high-resolution MS (ESI): m/z [M] $^+$ calc. for $C_{36}H_{56}NOP^+$: 549.4094; found: 549.4099. Anal. calcd. for $C_{36}H_{56}BrNOP$: C, 68.66; H, 8.96; N, 2.22%; found: C, 68.47; H, 8.89; N, 2.13%.

4. Conclusions

In this paper, we demonstrated that an *O*-acetyl nitroxide-protected paramagnetic diene can be used to synthesize phospholo[3,4-*c*] pyrrole scaffolds. Protected *O*-methyl pyrroline vinyl iodide can be converted to pyrroline nitroxide-diphenylphosphine oxide, for which reduction afforded pyrroline nitroxide-diphenylphosphine. A single-crystal X-ray diffraction study unambiguously supported the molecular structure. The pyrroline nitroxide diphenylphosphine can be converted into a hexadecyl phosphonium salt. In general, the proposed and adopted approaches could be used for a pyrroline nitroxide-containing phosphine- and pyrroline nitroxide-condensed *P*-heterocycle. Further synthetic and biological study of these compounds are in progress in our laboratory.

Supplementary Materials: Supplementary materials are available online. The 1H NMR and ^{13}C NMR, IR, MS spectra of novel compounds, and EPR spectra of compound 9 are available online. Table S1: Elemental analysis of new compounds synthesized, Figure S1: Preliminary biological data of compound 9 and cetyltriphenylphosphonium bromide (compound 10) compared to MITO-CP.

Author Contributions: Conceptualization, M.I. and T.K.; methodology, A.B., B.B., F.G. and J.J.; writing—original draft preparation, A.B., F.G. and T.K.; visualization, A.B. and F.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the European Union EFOP 3.6.1.-16-2016-00004.

Data Availability Statement: All data generated or analyzed during this study are included in this published article and CCDC contains the supplementary crystallographic data for 8 with deposition

number 2082286. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif accessed on 21 May 2021.

Acknowledgments: M.I. gives thanks for the Stipendium Hungaricum Scholarships. The authors give thanks to Mária Balog, and Kristina Kish (University of Pécs) for their technical assistance.

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

Sample Availability: Samples of compounds **2a**, **8**, and **9** are available from the authors.

References

1. Likhtenshtein, G.I. *Nitroxides: Brief History, Fundamentals, and Recent Developments*; Springer: Cham, Switzerland, 2020.
2. Ouari, O.; Gimes, D. (Eds.) *Nitroxides: Synthesis, Properties and Applications*; The Royal Society of Chemistry: Cambridge, UK, 2021.
3. Neiman, M.B.; Rozantsev, E.G.; Mamedova, Y.G. Free radical reactions involving no unpaired electrons. *Nature* **1962**, *196*, 472–474. [[CrossRef](#)]
4. Hideg, K.; Sár, P.C.; Hankovszky, H.O.; Tamás, T.; Jerkovich, G. Synthesis of new 3,4-disubstituted 2,5-dihydro-1*h*-pyrrol-1-ylloxyl spin-label reagents via allylic rearrangements. *Synthesis* **1993**, *25*, 390–394. [[CrossRef](#)]
5. Chalmers, B.A.; Morris, J.C.; Fairfull-Smith, K.E.; Grainger, R.S.; Bottle, S.E. A novel protecting group methodology for syntheses using nitroxides. *Chem. Commun.* **2013**, *49*, 10382–10384. [[CrossRef](#)] [[PubMed](#)]
6. Isbera, M.; Bognár, B.; Jekő, J.; Sár, C.; Hideg, K.; Kálai, T. Syntheses and reactions of pyrroline, piperidine nitroxide phosphonates. *Molecules* **2020**, *25*, 2430. [[CrossRef](#)] [[PubMed](#)]
7. Isbera, M.; Bognár, B.; Sár, C.; Jekő, J.; Kálai, T. Syntheses and utilizations of pyrroline-nitroxide- and tetrahydropyridine-nitroxide-based α -ketophosphonates, β -ketophosphonates, and a bisphosphonate. *Synth. Commun.* **2021**, *51*, 1353–1362. [[CrossRef](#)]
8. Rancurel, C.; Heise, H.; Köhler, F.H.; Schatzschneider, U.; Rentschler, E.; Vidal-Gancedo, J.; Veciana, J.; Sutter, J.-P. Spin transfer and magnetic interaction via phosphorus in nitronyl nitroxide radical-substituted triphenylphosphine derivatives. *J. Phys. Chem. A* **2004**, *108*, 5903–5914. [[CrossRef](#)]
9. Stipa, P.; Finet, J.P.; Le Moigne, F.; Tordo, P. β -Phosphorylated five-membered ring nitroxides: Synthesis and EPR study of 2-phosphonyl-4-(hydroxymethyl)pyrrolidine aminoxyl radicals. *J. Org. Chem.* **1993**, *58*, 4465–4468. [[CrossRef](#)]
10. Kálai, T.; Balog, M.; Jekő, J.; Hideg, K. Synthesis and reactions of symmetric paramagnetic pyrrolidine diene. *Synthesis* **1999**, *31*, 973–980. [[CrossRef](#)]
11. McCormack, W.B. Substituted Phosphacyclopentene Oxides and Process of Preparing Them. U.S. Patent 2,663,737, 22 December 1953.
12. Quin, L.D.; Middlemas, E.D.; Rao, S.N.; Miller, R.W.; McPhail, A.T. Synthesis and conformational properties of 3,8-phosphanedione 1-oxides. *J. Am. Chem. Soc.* **1982**, *104*, 1893–1900. [[CrossRef](#)]
13. Wang, J.Y.; Li, Q.J.; Xiao, Y.M.; Fu, B.; Qin, Z.H. Triphenylphosphonium (TPP)-based antioxidants: A new perspective on antioxidant Design. *ChemMedChem* **2020**, *15*, 404–410. [[CrossRef](#)] [[PubMed](#)]
14. Zielonka, J.; Joseph, J.; Sikora, A.; Hardy, M.; Ouari, O.; Vasquez-Vivar, J.; Cheng, G.; Lopez, M.; Kalyanaraman, B. Mitochondria-targeted triphenylphosphonium-based compounds: Syntheses, mechanism of action, and therapeutic and diagnostic applications. *Chem. Rev.* **2017**, *117*, 10043–10120. [[CrossRef](#)] [[PubMed](#)]
15. Dhanasekaran, A.; Kotamraju, S.; Karunakaran, C.; Kalivendi, S.V.; Thomas, S.; Joseph, J.; Kalyanaraman, B. Mitochondria superoxide dismutase mimetic inhibits peroxide-induced oxidative damage and apoptosis: Role of mitochondrial Superoxide. *Free Rad. Biol. Med.* **2005**, *39*, 567–583. [[CrossRef](#)] [[PubMed](#)]
16. Kálai, T.; Bognár, B.; Jekő, J.; Hideg, K. Synthesis of new, paramagnetically modified heterocycles. *Synthesis* **2006**, *38*, 2573–2579.
17. Fritzsche, H.; Hasserodt, U.; Korte, F. Reduktion organischer verbindungen des fünfwertigen phosphors zu phosphinen, II. reduktion tertiärer phosphinoxyde zu tertiären phosphinen mit trichlorsilan. *Chem. Ber.* **1965**, *98*, 171–174. [[CrossRef](#)]
18. Kovács, T.; Keglevich, G. The reduction of tertiary phosphine oxides by silanes. *Curr. Org. Chem.* **2017**, *21*, 569–585. [[CrossRef](#)]
19. Sheldrick, G.M. A short history of SHELX. *Acta Cryst.* **2008**, *A64*, 112–122. [[CrossRef](#)] [[PubMed](#)]
20. Westrip, S.P. publCIF: Software for editing, validating and formatting crystallographic information files. *J. Appl. Crystallogr.* **2010**, *43*, 920–925. [[CrossRef](#)]
21. Mercury, C.F.; Macrae, P.R.; Edgington, P.M.E.; Pidcock, G.P.; Shields, R.; Taylor, M.T.; van de Streek, J. Mercury: Visualization and analysis of crystal structures. *J. Appl. Crystallogr.* **2006**, *39*, 453–457. [[CrossRef](#)]
22. Spek, A.L. Single-crystal structure validation with the program PLATON. *J. Appl. Crystallogr.* **2003**, *36*, 7–13. [[CrossRef](#)]
23. Groom, C.R.; Bruno, I.J.; Lightfoot, M.P.; Ward, S.C. The cambridge structural database. *Acta Cryst.* **2016**, *B72*, 171–179. [[CrossRef](#)] [[PubMed](#)]