Synthesis and Single-Crystal X-ray Investigation of 4-Azido-2-(triphenylphosphinimino)-3,5,6-trifluorobenzonitrile: A Chromogenic Nitrene Precursor for Photolabeling

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Introduction

Development of new photolabeling agents with improved photochemical properties has been a long sought goal for molecular biologists and chemists.[1−9] The minimum characteristics for a UV-activated photolabeling agent include high quantum efficiencies for forming the reactive precursor and high reactivity for insertion into unactivated bonds.[10,11] Although perfluoro azides are projected to be potential photolabeling agents,[12−14] most require photoactivation in the spectral region where proteins and nucleic acids[15] also absorb light.

One goal of our work is to develop chromogenic photoreactive nitrene precursor that can be activated by irradiation at wavelengths beyond the protein absorption tail (i.e., $\lambda_{max} > 320$ nm) since photolysis at these wavelengths preclude the possibility of denaturing of proteins by radiation[16] and can compensate for a lower quantum yield of the photoprobe. The spectral separation of the photoprobe and its photolysis products from the absorption region of the biomolecule also provides a spectral handle for monitoring and separating the photolabeled biomolecules from the nonlabeled products using high-performance liquid chromatography (HPLC) with a multiwavelength detector.

A second goal is to introduce bifunctional character to the photoprobe by attaching chelating agents for the incorporation of transition metals. Ligands with phosphorus-nitrogen backbones are of particular interest by virtue of their chelation potential with a variety of transition metals (e.g., Pd, Re, Rh, etc.), the parallel radioisotopes of which are useful in radiopharmaceuticals.[17] In continuation of our earlier studies on the development of photolabeling agents,[18−23] we report here the synthesis and single crystal X-ray structure of 4-azido-2-(triphenylphosphinimino)-3,5,6-trifluorobenzonitrile, **2**, a chromogenic nitrene precursor, and the complexation of **2** with Pd(II). The fundamental coordination properties of the Pd(II) complex system can be extended to the corresponding analogues with ^{109}Pd, a β-emitting therapeutic radionuclide for use in radioimmunotherapy.

Experimental Section

All reactions and other manipulations were carried out under an atmosphere of dry nitrogen or under vacuum. Solvents were prepurified, dried and distilled under nitrogen prior to use. ^{1}H, ^{31}P, and ^{19}F NMR spectra were recorded on a Bruker WH-300 instrument for samples in $CDCl_3$ solvent. ^{1}H NMR chemical shifts are reported in ppm, downfield from external standard, $SiMe_4$. The ^{31}P NMR chemical shifts are reported with respect to 85% H_3PO_4 as an external standard and for ^{19}F NMR, trifluorotoluene is used as an external standard. Reagents such as $PdCl_2(PhCN)_2$ and perfluorobenzonitrile were purchased from Aldrich Chemical Co. All elemental analysis were done by Oneida Research Services, Inc., Whitesboro, NY. FTIR spectra were taken in a Mattson Galaxy 3000 spectrophotometer instrument using a Nujol mull.

Synthesis of 4-Azido-2-(triphenylphosphinimino)-3,5,6-trifluorobenzonitrile, 2. A two-necked flask, equipped with a nitrogen inlet adapter and an addition funnel was charged with dry methylene chloride (100 mL) and 4-azido-2,3,4,5-tetrafluorobenzonitrile, **1** (5 g, 21.3 mmol). A solution of (trimethylsilyl)triphenylphosphinimine R_3PNT (R = Ph and T = $(CH_3)_3Si$, 8.17 g, 23.4 mmol), prepared by refluxing triphenylphosphine with trimethylsilyl azide,[24] was slowly added dropwise at 0 °C and stirred for an hour during which the reaction was monitored by ^{31}P NMR spectroscopy. The solvent was evaporated under the atmosphere of nitrogen and the solid was redissolved in dry acetonitrile and evaporated slowly to give yellow crystals **2** in 90% yield. mp: 126 °C. Anal. Calcd for $C_{25}H_{15}N_5F_3P$: C, 63.43, H, 3.19, N, 14.79, Found: C, 63.23, H, 3.41, N, 14.83. ^{1}H NMR: δ 7.0−8.0 (m, 15H, aromatic protons). ^{31}P NMR: δ 9.8(s). ^{19}F NMR: δ −73.6 (m, 1F), −74.6 (m, 1F and −99.4 (m, 1F) IR: 2250, 2122, 1628, 1377, 1258, 1200, 1109, 1045, 719, 525 cm^{-1} UV: 260 nm (ϵ =15 340 M^{-1} cm^{-1}), 348 nm. (ϵ =1632 M^{-1} cm^{-1}).

Synthesis of [4-Azido-2-triphenylphosphinimino-3,5,6-trifluorobenzonitrile−PdCl$_2$], 3. A solution of the $PdCl_2(PhCN)_2$ precursor

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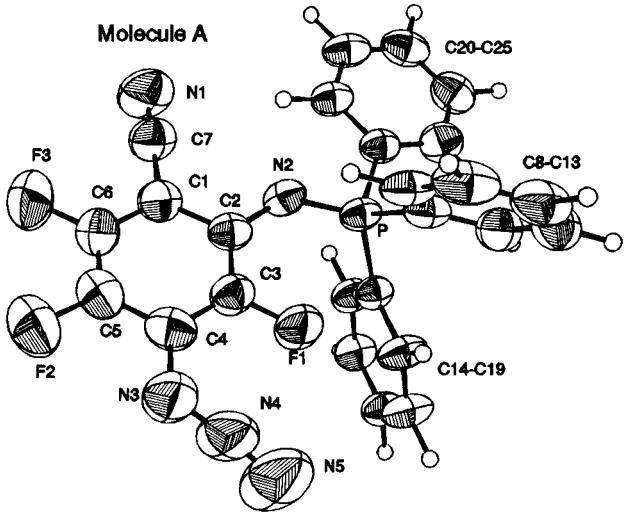

Figure 1. ORTEP plot for orientation A of 4-azido-2-(triphenylphosphinimino)-3,5,6-trifluorobenzonitrile, **2**.

Table 1. Crystallographic Data for Compound **2**

formula	$C_{25}H_{15}N_5F_3P$	*M*	473.4
cryst size/mm	0.10 × 0.25 × 0.50	cryst syst	monoclinic
space group	$P2_1/c$	*a*/Å	16.425(7)
b/Å	18.653(5)	*c*/Å	14.861(9)
β/deg	100.490(10)	*U*/Å^3	4477(4)
F(000)	1936	*Z*	8
D_c/g cm^{-3}	1.405	$2\theta_{max}$/deg	120
μ/cm^{-1}	15.1	*hkl* ranges	−18 to +18, 0−20, 0 to −16
no. of tot. data	6919		
R^a	0.057	no. of unique data	6623
		R' [b]	0.084

[a] $R = \sum(|F_o| - |F_c|)/\sum|F_o|$. [b] $R' = [\sum w(|F_o| - |F_c|)^2/\sum|F_o|^2]^{1/2}$; $w^{-1} = [\sigma^2|F_o| + 0.0008(F_o)^2]$.

in dichloromethane (0.380 g, 1 mmol) was slowly added dropwise to a solution of **2** (0.461 g, 0.97 mmol) also taken in dichloromethane at 25 °C. The reaction mixture was stirred for 10−15 min, during which time it was monitored by ^{31}P NMR spectroscopy. The solvent was partially removed under vacuum and *n*-hexane was added to induce precipitation. The precipitate was washed with *n*-hexane (3 × 20 mL) to remove the benzonitrile, then dried in vacuum to give a yellow crystalline Pd complex in 75% yield. Mp: 265 °C dec. Anal. Calcd for $C_{25}H_{15}N_5F_3PPdCl_2$: C, 46.14, H, 2.32, N, 10.76. Found: C, 46.56, H, 2.61, N, 10.53. ^{1}H NMR: δ 7.0−7.8, (m, 15H, aromatic protons). ^{31}P NMR: δ 35.6. ^{19}F NMR: δ −59.3 (m, 1F), −65.3 (m, 1F) −86.2 (m, 1F) IR: 2228, 2120 , 1622, 1520, 1480, 1250, 1109, 721 cm^{-1}. UV: 260 (ϵ = 14 543 M^{-1} cm^{-1}), 348 (ϵ = 9567 M^{-1} cm^{-1}).

Results and Discussion

The nucleophilic substitution of **1** by substituted phosphinimine resulted in the preferential ortho substitution product as evidenced by ^{19}F NMR which shows three multiplets for the three fluorines instead of the two multiplet AA^{1}XX^1 pattern for the symmetrical parent azide.[14,20] The ^{31}P NMR spectrum shows a singlet at 9.8 δ shifted from 1.6 δ for the phosphinimine derivative **2**. This compound is an air stable solid that readily dissolves in ethanol. The presence of a strong IR band at 2123 cm^{-1} indicates that the azide moiety is still present. The interesting aspect of this nucleophilic substitution is the shifting of λ_{max} from 270 nm for **1** to 348 nm in **2**. This permits selective irradiation of the probe in presence of biomolecules.

X-ray crystal structure of $C_{25}H_{15}N_5F_3P$, 2. To obtain a conclusive proof for the nondisturbance of the azide moiety by attachment of the ligating system, a single-crystal X-ray stuctural analysis of phosphinimine was carried out. The ORTEP[25,26] for the two orientations is shown in Figures 1 and 2. The compound crystallizes in monoclinic form $P2_1/c$ and has two crystallographically independent orientations in the solid state. The salient crystallographic data are summarized in Table 1. Selected bond lengths and bond angles are tabulated in Table 2. Typical bond lengths of the azide moiety along with bond angles indicate that they are in the normal range reported for the other azides.[19]

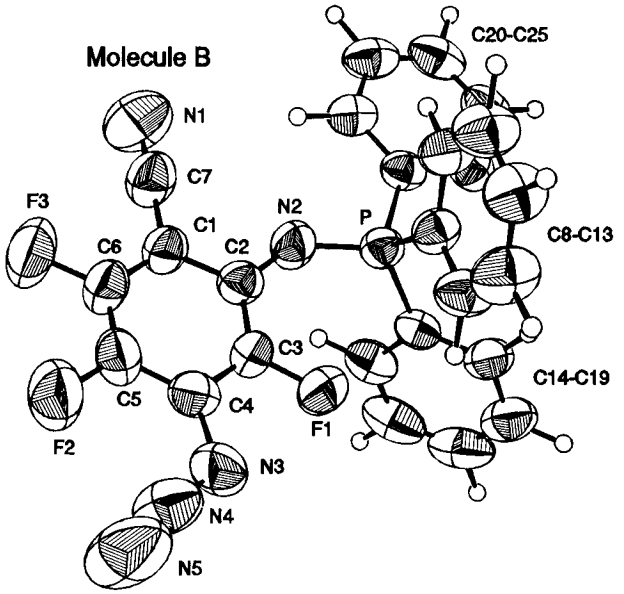

Figure 2. ORTEP plot for orientation B of **2**.

Table 2. Selected Bond Lengths and Angles for Compound **2**

	Lengths (Å)		
PA−N2A	1.566(3)	PB−N2B	1.578(3)
PA−C8A	1.806(3)	PB−C8B	1.796(4)
PA−C14A	1.798(3)	PB−C14B	1.800(4)
PA−C20A	1.807(3)	PB−C20B	1.800(4)
N1A−C7A	1.121(5)	N1B−C7B	1.166(6)
N2A−C2A	1.354(4)	N2B−C2B	1.361(5)
N3A−N4A	1.196(5)	N3B−N4B	1.215(5)
N3A−C4A	1.406(5)	N3B−C4B	1.393(5)
N4A−N5A	1.130(6)	N4B−N5B	1.144(6)
F1A−C3A	1.352(4)	F1B−C3B	1.352(4)
F2A−C5A	1.353(5)	F2B−C5B	1.355(4)
F3A−C6A	1.344(4)	F3B−C6B	1.352(4)
C1A−C2A	1.425(5)	C1B−C2B	1.404(5)
C1A−C6A	1.364(5)	C1B−C6B	1.377(6)
C1A−C7A	1.435(5)	C1B−C7B	1.406(6)
C2A−C3A	1.390(5)	C2B−C3B	1.399(5)
C3A−C4A	1.376(5)	C3B−C4B	1.372(5)
C4A−C5A	1.393(6)	C4B−C5B	1.389(5)
C5A−C6A	1.354(6)	C5B−C6B	1.353(6)
	Angles (deg)		
N2A−PA−C8A	114.65(16)	N2B−PB−C8B	114.61(17)
N2A−PA−C14A	117.03(15)	N2B−PB−C14B	115.44(17)
N2A−PA−C20A	103.90(15)	N2B−PB−C20B	104.41(17)
PA−N2A−C2A	137.81(24)	PB−N2B−C2B	133.0(3)
N4A−N3A−C4A	118.7(3)	N4B−N3B−C4B	116.5(4)
N3A−N4A−N5A	169.6(4)	N3B−N4B−N5B	169.2(5)
N2A−C2A−C1A	118.1(3)	N2B−C2B−C1B	118.5(3)
N2A−C2A−C3A	127.4(3)	N2B−C2B−C3B	126.6(3)

The presence of a nitrogen on the phosphinimine and another on the nitrile group within the same molecule opens up a possibility of using them for chelation to transition metals presumably through the formation of six membered ring (Scheme 1). For example, **2** reacted with $PdCl_2(PhCN)_2$ to produce a new metallacyclic compound **3** containing Pd(II) in

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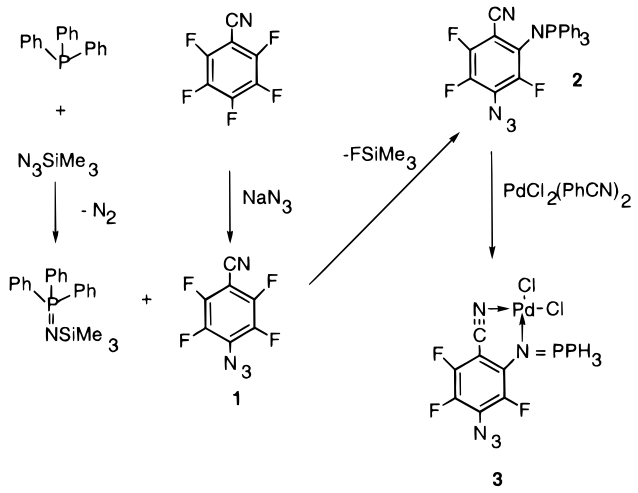
Scheme 1



high yield(>75%). The chemical constitution of **3** as established by C, H, and N analytical data indicates that the complex has one metal center per phosphinimine unit. The additional support was provided by ^{31}P NMR spectrum, which shows a singlet signal at 36.4 δ with a moderate shift of 26 δ when compared to the ligand **2**. ^{19}F NMR of the Pd complex shows all 3 fluorine signals being shifted downfield compared to the parent ligand. Comparison of the ν(C≡N) stretching frequency of the free ligand with the corresponding Pd(II) complex shows a shift from 2250 cm^{-1} to 2228 cm^{-1}. This observation, along with the C, H, and N analytical data indicate the formation of the Pd complex (Scheme 1). A strong IR band at 2125 cm^{-1} is in a range similar to that for other azides reported[13] and indicates that the azide moiety has not been perturbed during chemical manipulations.

Photolysis of the precursor azide **1** (Scheme 1) produces a very high yield of singlet nitrene intermediates capable of inserting into C-H bonds.[20] Screening for the ability of the ligand **2** to retain this favorable characteristic was carried out with the pyridine trapping technique[27,28] which converts singlet nitrenes into stable pyridinium ylides. These results are shown in Figure 3, which compares the characteristic ylide formation from a high yield nitrene precursor **1** to the ligand **2**. The characteristic absorption of the pyridinium ylide at 394−416 nm clearly indicates that **2** also produces singlet nitrene intermediates upon photolysis, but with a much lower quantum yield than the parent perfluoroazide.

Conclusion

The modification of perfluorocyanoazide through the nucleophilic substitution by phosphinimine resulted a considerable bathochromic shift to 350 nm, leading to a new chromogenic nitrene precursor **2**. This permits the selective electronic excitation of the chromophore avoiding possible photo damage to biomolecules. The newly synthesized photoprobe crystallizes in $P2_1/c$ and shows the azide moiety to be intact. The ability of **2** to form singlet nitrene intermediates upon photolysis coupled with its ability to form a Pd(II) complex demonstrates the bifunctional character to the new photoprobe. The high yield for formation of the Pd(II) complex suggests that **2** should be useful for incorporating the ^{109}Pd radionuclide. The reaction of **2** with ^{109}Pd (as $^{109}PdCl_4^{2-}$) forms a complex which has been characterized by paper and thin layer chromatography by radioanalytical imaging AMBIS.[29] Although the quantum yield for singlet nitrene formation with this compound is low, the ability to excite the photoprobe at wavelengths which do not perturb the biomolecule provides the flexibility of a longer photolysis time to achieve labeling. Further investigations into developing bifunctional photolabeling agents will focus on increasing the singlet nitrene yield while retaining the complexation ability of the ligand.

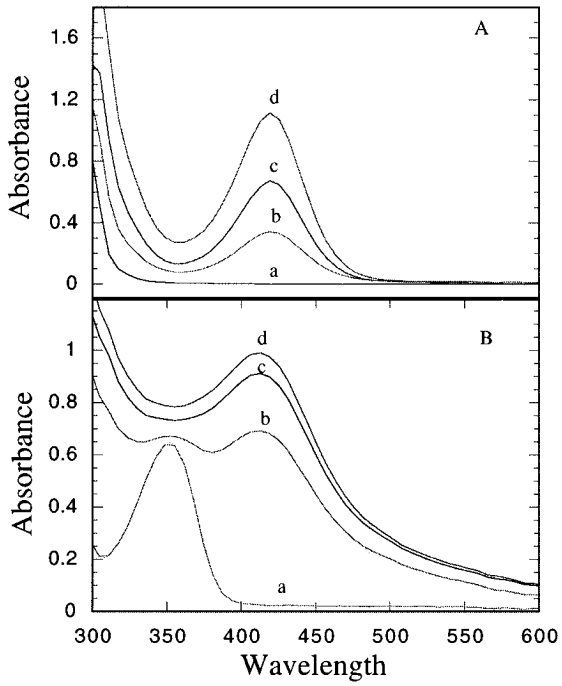


Figure 3. Spectral changes of **1** and **2** as a function of exposure time to the full beam of a 200 W Hg lamp filtered to remove wavelengths below 320 nm. The solvent was 1:1 pyridine−methylene chloride. Key: (A) exposure time for **1** (λ_{max} = 265 nm, spectra below 300 nm were masked by pyridine absorption) was (a) 0, (b) 10, (c) 20, and (d) 80 s; (B) photolysis of **2** was (a) 0, (b) 1, (c) 2, and (d) 5 min.

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Supporting Information Available: Tables giving crystallographic details, atomic coordinates, thermal parameters, bond angles, torsion angles, and bond lengths for **2** (9 pages). Ordering information is given on any current masthead page.

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