Chemistry of Bifunctional Photoprobes.[1] 3. Correlation between the Efficiency of CH Insertion by Photolabile Chelating Agents and Lifetimes of Singlet Nitrenes by Flash Photolysis: First Example of Photochemical Attachment of ^{99m}Tc−Complex with Human Serum Albumin

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Systematic functionalization of perfluoroaryl azides with chelating agents capable of complexing transition metals produces a new class of bifunctional photolabile chelating agents (BFPCAs). The strategy to shield the azide functionality from the electronic and steric influence of the electron-rich metal Pd through ester and amide bridges raised CH insertion efficiency to unprecedented levels (>92%) in a model solvent (cyclohexane). In contrast, perfluoroaryl azides attached to chelating agents via hydrazones show no significant CH insertion in cyclohexane upon photolysis. Measurements of the lifetimes of the singlet nitrenes derived from these agents by flash photolysis techniques correlate well with the efficiency of CH insertion by demonstrating longer lifetimes (10−50 times) for singlet nitrenes derived from azidotetrafluorinated esters and amides compared with the related hydrazones, which failed to yield significant CH insertion. A representative BFPCA **12** is chelated to diagnostic radionuclide ^{99m}Tc and covalently attached to human serum albumin via photochemical activation extending the favorable bimolecular insertion characteristics of BFPCA to tracer level concentrations in buffer conditions. Flash photolysis experiments correlate singlet nitrene lifetimes with the efficiency of intermolecular insertion reactions. This work provides new photo-cross-linking technology, useful in radiodiagnostics and radiotherapy in nuclear medicine.

Introduction

Development of methods for covalent modification of biomolecules produces new tools in biochemistry and medicine.[2−4] The technique of photolabeling has been a valuable method for arming monoclonal antibodies,[5] receptors,[6] and organic substrates carrying toxins or radionuclides,[7] resulting in the development of new diagnostic and therapeutic agents. Although several reported heterobifunctional photolabeling reagents incorporate aryl azide and ^{3}H or ^{125}I radiolabels at termini,[8−10] applications in nuclear medicine and antibody-target cancer therapy require incorporation of transition metals and their analogous radioisotopes.[11] In particular,

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(1) For Part 2, see Pandurangi, R. S.; Lusiak, P.; Kuntz, R. R.; Sun, Y.; Weber, K. T. Chemistry of Bifunctional Photoprobes: Part 2. Chemical and Photochemical Modification of Angiotensin Converting Enzyme (ACE) Inhibitors: Implications in the Development of Cardiac Radionuclide Imaging Agents. *Bioorg. Chem.* **1997**, *25*, 77.

(2) (a) Kozak, R. W.; Raubitschek, A.; Mirzadeh, S.; Brechbiel, M. W.; Junghauns, R.; Gansow, O. A.; Waldmann, T. A. *Cancer Res.* **1989**, *49*, 2639. (b) Mathias, C. J.; Sun, Y.; Connett, J. M.; Philpott, G. W.; Welch, M. J.; Martell, A. E. *Inorg. Chem.* **1990**, *29*, 1475 and references therein. (c) Paik, C. H.; Murphy, P. R.; Eckelman, W. A.; Volkert, W. A.; Reba, R. C. *J. Nucl. Med.* **1983**, *24*, 932. (d) Cacheris, W. P.; Nickle, S. K.; Sherry, A. D. *Inorg. Chem.* **1987**, *26*, 958.

(3) (a) Lever, S. Z.; Baidoo, K. E.; Mahmood, A. *Inorg. Chim. Acta* **1990**, *176*, 183. (b) Brechbiel, M. W.; Gamsow, O. A.; Atcher, R. W.; Schlom, J.; Esteban, J.; Simpson, D. E.; Colcher, D. *Inorg. Chem.* **1986**, *25*, 2772.

(4) (a) Cummins, C. H.; Rutter, E. W.; Fordyce, W. A. *Bioconjugate Chem.* **1991**, *2*, 180. (b) Moi, M. K.; Meares, C. F.; DeNardo, S. J. *J. Am. Chem. Soc.* **1988**, *110*, 6266. (c) Meares, C. F.; Goodwin, D. A. *J. Protein Chem.* **1984**, *3*, 215. (d) Baidoo, K. G.; Scheffel, U.; Lever, S. Z. *Cancer Res.* **1990**, *50*, 799.

(5) Mirzadeh, S.; Brechbiel, M. W.; Atcher, R. W.; Gansow, O. A. *Bioconjugate Chem.* **1990**, *1*, 59.

(6) (a) Cavalla, D.; Neff, N. H. *Biochem. Pharmacol.* **1985**, *34*, 1821. (b) Haley, B. E. *Methods Enzymol.* **1991**, *200*, 477. (c) Neilson, P. E. In *Photochemical Probes in Biochemistry*; Neilson, P. E., Ed.; NATO ASI Series C, 272; Kluver Academic Publishers: Boston, 1989. (d) Lewis, C. T.; Haley, B. E.; Carlson, G. M. *Biochemistry* **1989**, *28*, 9248. (e) Bayley, H.; Staros, J. W. Photoaffinity Labeling and Related Techniques. In *Azides and Nitrenes, Reactivity and Techniques*; Scriver, E. F. V., Ed.; Academic Press: New York, 1984; Chapter 9, p 433. (f) Bayley, H. Reagents for Photoaffinity Labeling. In *Photogenerated Reagetns in Biochemistry and Molecular Biology*, Work, T. S.; Burdon, R. H., Eds.; Elsevier: Amsterdam, 1983; Chapter 3, p 26. (g) Brunner, J. *Annu. Rev. Biochem.* **1981,** *62*, 483. (h) Brunner J. *Trends Biochem. Sci.* **1982**, *35*, 44.

(7) (a) Mach, R. H.; Kung, H. F.; Jungwiwattanaporn, P.; Guo, Y.-Z. *Tetrahedron Lett.* **1989**, *30*, 4069. (b) Baidoo, K. F.; Lever, S. Z. *Bioconjugate Chem.* **1990**, *1*, 132. (c) Meares, C. F.; McCall, M. J.; Reardan, D. T.; Goodwin, D. A.; Diamanti, C. I.; McTigue, M. *Anal. Biochem.* **1984**, *142*, 68. (d) Fritzberg, A. R.; Abrams, P. G.; Baumier, P. L.; Kasina, S.; Morgan, A. C., Jr.; Rao, T. N.; Reno, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **85**, 4025. (e) Baidoo, K. E.; Lever, S. Z. *Tetrahedron Lett.* **1990**, *31*, 5701 (f) Meares, C. F.; Goodwin, D. A. *J. Protein Chem.* **1984**, *3*, 215. (g) Miki, K.; Kubota, K.; Kokudu, N.; Inoue Y.; Bandai, Y.; Makuuchi M. *J. Nucl. Med.* **1997**, *38*, 1798.

(8) Katzenellenbogen, J. A.; Myers, H. N.; Johnson, H. J.; Kempton, R. J.; Carlson, D. E. *Biochemistry* **1977**, *16*, 1964−1976.

(9) Garg, S.; Garg, P. K.; Zalutsky, M. R. *Bioconjugate Chem.* **1991**, *2*, 50.

(10) Khawli, L. A.; Kasis, A. I. *Nucl. Med. Biol.* **1989**, *16*, 727.

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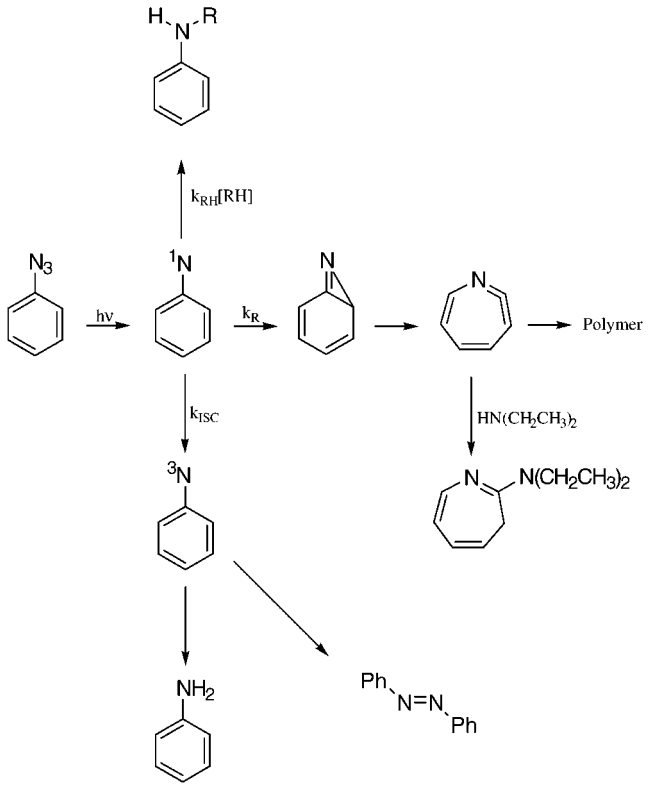
covalent attachment of ^{99m}Tc-based bifunctional photolabile chelating agent (BFPCA) to biomolecules produces new diagnostic probes.

Perfluoroaryl azides constitute an important class of photolabeling agents[12−15] that are superior to conventional aryl azide or nitroaryl azide reagents. Photolysis of nonfluorinated aryl azides generates singlet aryl nitrenes that are consumed rapidly by intramolecular reactions to yield secondary intermediates (ketenimines, triplet nitrenes) and which, unlike their polyfluorinated analogues, do not insert into unactivated CH bonds (Scheme 1).[16,17]

The efficiency of CH insertion by bifunctional metalated photolabels into antibodies is the key requirement for successful application of this method in targeted cancer therapy.[18] Labeling in the hydrophobic regions of the antibody, primarily by insertion reactions, facilitates the preservation of immunoreactivity, which is essential for transport of radioactivity to the antigen sites (tumor).[19] Recently, we reported the high retention of immunoreactivity of the B72.3 antibody (>97%) after photolabeling using a ^{14}C-labeled photoprobe.[20] The next milestone is the demonstration of efficient photoconjugation by BFPCAs carrying ^{99m}Tc and other useful transition metals (e.g ^{109}Pd, ^{105}Rh, ^{186}Re, etc.).

To our knowledge, the intermolecular insertion chemistry of bifunctional photolabels carrying transition metals in model solvents and proteins is relatively unexplored,[21] although introduction of an electron-rich metal such as Pd has been shown to dramatically[22] reduce the

Scheme 1



CH insertion yield of perfluoroaryl nitrenes. The design of useful photoprobes requires an understanding of the effect of substituents on the rate of rearrangement, intersystem crossing, and the bimolecular reactivity of singlet aryl nitrenes which has prompted this investigation. Modifications of the tether linking perfluoroaryl azides by transition metals may, therefore, affect both the quantum yield for singlet nitrene formation and the efficiency of the insertion reactions. Herein we report the effect of electronic and steric separation of the photolabile group and the metal chelate on the CH insertion yield in cyclohexane and its correlation with singlet nitrene lifetimes, as measured by laser flash photolysis techniques. Our data show a direct correlation between nitrene C−H insertion efficiency and singlet lifetime. We report the largest CH insertion yield of a singlet aryl nitrene in cyclohexane ever observed (>92%). We also extend the results in model organic solvents to buffer conditions at tracer level concentrations by covalent attachment of the ^{99m}Tc-complex of BFPCA to human serum albumin (HSA). This new technology may be useful for hepatic imaging.[7g]

Results

Synthesis. The general synthetic targets are heterobifunctional photolabel agents with a specific ligating

(11) (a) Jurisson, S.; Berning, D.; Jia, W.; Ma, D. *Chem. Rev.* **1993**, *93*, 1137. (b) Meares, C. F. *Nucl. Med. Biol.* **1986**, *13*, 311. (c) Brechbiel, M. W.; Gansow, O. A. *Bioconjugate Chem.* **1991**, *2*, 187. (d) Adams, G. P.; DeNardo, G. L. *J. Nucl. Med. Biol.* **1989**, *16*, 587. (e) Meares, C. F.; McCall, M. J.; Reardon, D. T.; Goodwin, D. A.; Diamanti, M. W.; Gansow, O. A.; Atcher, R. W.; Schlom, J.; Esteban, J.; Simpson, D. E.; Colcher, D. *Inorg. Chem.* **1986**, *25*, 2772. (f) Brechbiel, M. W.; Gansow, O. A. *Bioconjugate Chem.* **1991**, *2*, 187.

(12) (a) Hibert, F. K.; Isabelle, K.; Goeldner M. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1296. (b) Fleming, S. A. *Tetrahedron* **1995**, *51*, 12479.

(13) (a) Leyva, E.; Young, M. J. T.; Platz, M. S. *J. Am. Chem. Soc.* **1986**, *108*, 8307. (b)Young, M. J. T.; Platz, M. S. *Tetrahedron Lett.* **1989**, *30*, 2199 (c) Leyva, E.; Munoz, D.; Platz, M. S. *J. Org. Chem.* **1989**, *54*, 5938. (d) Poe, R.; Schnapp, K.; Young, M. J. T.; Grayzar, J.; Platz, M. S. *J. Am. Chem. Soc.* **1992**, *114*, 5054. (e) Young, M. J. T.; Platz, M. S. *J. Org. Chem.* **1991**, *56*, 6403. (f) Levya, E.; Munoz, D.; Platz, M. S. *J. Org. Chem.* **1989**, *54*, 5938.

(14) (a) Keana, J. F. W.; Cai, S. X. *J. Org. Chem.* **1990**, *55*, 3640. (b) Keana, J. F. W.; Cai, S. X. *J. Fluorine Chem.* **1989**, *43*, 151. (c) Cai, S. X.; Glenn, D. J.; Keana, J. F. W. *J. Org. Chem.* **1992**, *57*, 1299. (c) Keana, J. F. W.; Cai, S. X. *J. Fluorine Chem.* **1988**, *43*, 151. (d) Cai, S. X.; Keana, J. F. W. *Tetrahedron Lett.* **1989**, *30*, 5409. (e) Cai, S. X.; Glenn, D. J.; Gee, K. R.; Yan, M.; Cotter, R. E.; Reddy, N. L.; Weber, E.; Keana, J. F. W. *Bioconjugate Chem.* **1993**, *4*, 545.

(15) (a) Pinney, K. G.; Carlson, K. E.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. *J. Biochem.* **1991**, *30*, 2421. (b) Pinney, K. G.; Katzenellenbogen, J. A. *J. Org. Chem.* **1991**, *56*, 3125. (c) Bergmann, K. E.; Carlson, K. E.; Katzenellenbogen, J. A. *Bioconjugate Chem.* **1994**, *5*, 141. (d) Pinney, K. C.; Katzenellenbogen, J. A. *J. Org. Chem.* **1991**, *56*, 3125.

(16) (a) Schuster, G. B.; Platz, M. S. In *Advances in Photochemistry*; Volman, D.; Hammond, G.; Neckers, D., Eds; John Wiley: New York, 1992; Vol. 17, p 69. (b) Scriven E. F. V. In *Reactive Intermediates*; Abramovitch, R. A., Ed.; Wiley: New York, 1981; Vol. 2, Chapter Moss, R. A., Chapter 8. (c) Reiser, A.; Wagner, H. M. *The Chemistry of the Azido Group*; Patai, S., Ed., Wiley: New York, 1971. (d) Schrock, A. K.; Schuster, G. B. *J. Am. Chem. Soc.* **1984**, *66*, 5228.

(17) (a) Shrock. A. K.; Schuster, G. B. *J. Am. Chem. Soc.* **1984**, *106*, 5228. (b) Leyva, E., Platz, M. S.; Persy, G.; Wirz, J. *J. Am. Chem. Soc.* **1986**, *108*, 3783.

(18) (a) Waldman, T. A. *Science* **1991**, *252*, 1657. (b) Winter G.; Milstein C. *Nature* **1991**, *349*, 293.

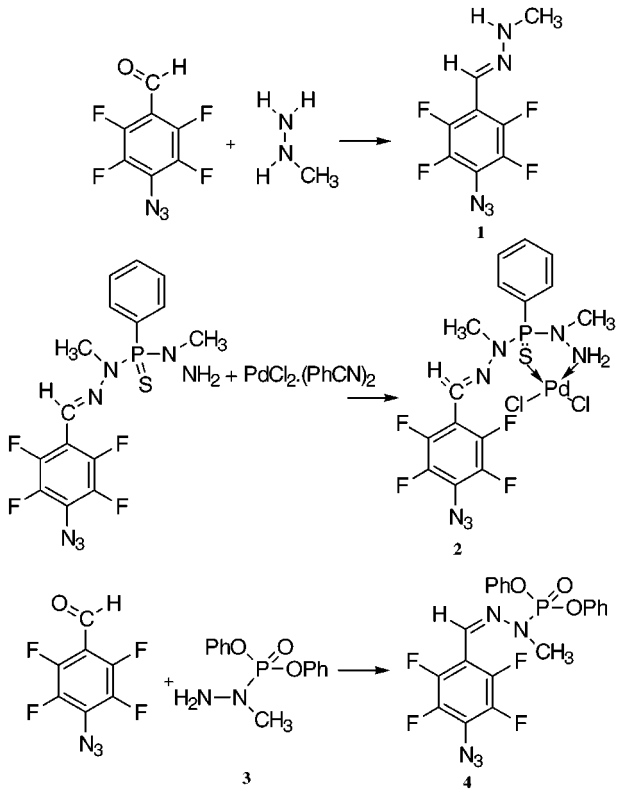
(19) Buchsbaum D. J.; Lawrence T. S. *Antibody, Immunoconjugates Radiopharm.* **1991**, *4*, 245.

(20) Pandurangi, R. S.; Karra, S. R.; Kuntz, R. R.; Volkert, W. A. *Photochem. Photobiol.* **1996**, *65*, 106.

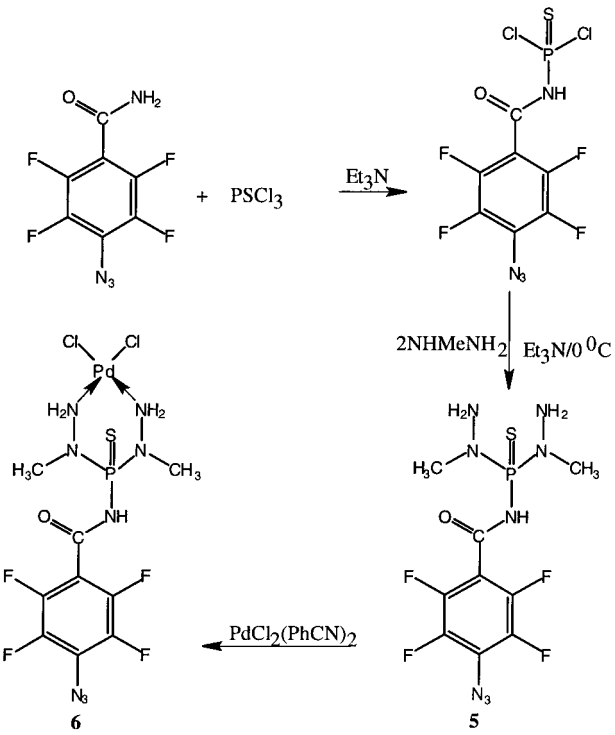
(21) For a recent review see: (a) Pandurangi, R. S.; Karra, S. R.; Kuntz, R. R.; Volkert, W. A. *Photochem. Photobiol.* **1997**, *66*, 208. (b) Pandurangi, R. S.; Kuntz, R. R.; Volkert, W. A.; Barnes, C. L.; Katti, K. V. *J. Chem. Soc., Dalton Trans.* **1995**, 565. (c) Pandurangi, R. S.; Karra, S. R.; Kuntz, R. R.; Volkert, W.A. *Bioconjugate Chem.* **1995**, *6*, 630. (d) Pandurangi, R. S.; Katti, K. V.; Barnes, C. L.; Volkert, W. A.; Kuntz, R. R. *Chem. Commun.* **1994**, 1841. (e) Pandurangi, R. S., Kuntz, R. R., and Volkert, W. A. *App. Rad. Isotopes*, **1995**, *46*, 233. (f) Pandurangi, R. S.; Katti, K. V.; Barnes, C. L.; Volkert, W. A.; Kuntz, R. R. *Inorg. Chem.* **1996**, *35*, 3716.

(22) Pandurangi, R. S.; Karra, R. S.; Katti, K. V.; Kuntz, R. R.; Volkert, W. A. *J. Org. Chem.* **1997**, *62*, 2798.

Scheme 2



Scheme 3



framework connected via different tethers. Phosphorus hydrazides were chosen as ligating moieties because they had been used successfully for chelation to diagnostic and therapeutic radiometals[23,24] (^{99m}Tc and ^{109}Pd). A simple and convenient method that links the photolabel and the chelating framework is Schiff base coupling between perfluoroaryl azidobenzaldehyde and the amine group of methyl hydrazine and similarly, with mono- and bishydrazidophosphorus compounds. Dropwise addition of an equimolar solution of 4-azidotetrafluorobenzaldehyde in absolute ethanol to a solution of methyl hydrazine or diphenoxyphosphorylmethyl hydrazine **3** in ethanol at room temperature forms hydrazones **1** and **4**, respectively, in high yields (>90%, Scheme 2). Coupling perfluoroaryl azidobenzaldehyde with bishydrazidophosphorus sulfide was conducted at −70 °C to increase the selectivity of monosubstitution, which is reacted with $PdCl_2$·$(PhCN)_2$ to give Pd complex 2 as reported earlier.[21b]

An alternate linker design introduces an electron-withdrawing group para to the azide moiety in the form of an ester or amide group. However, in this case the reactivity of the P−Cl bond in $PSCl_3$ toward nucleophilic substitution is controlled by the less basic amide group on the azidotetrafluorobenzamide (Scheme 3). For example, harsh reaction conditions (refluxing for hours) were necessary for $PSCl_3$ to react with azidotetrafluorobenzamide in the presence of excess Et_3N for the formation of monosubstituted product. Addition of 2 equiv of methyl hydrazine at 0 °C in situ in the presence of excess of Et_3N produces **5** in good yield. ^{31}P NMR of **5** shows a single phosphorus signal at δ 67 and a clean doublet (^3J (P−H) = ∼11−12 Hz) at δ 2.97. ^{1}H NMR reveals the coupling of methyl protons with phosphorus indicating the incorporation of methyl hydrazine.[23a]

The ester- and amide-linked reagents were prepared as follows. Treatment of pentafluorobenzoyl chloride with an excess of ethylene glycol in tetrahydrofuran (THF), followed by refluxing overnight, resulted in the formation of monosubstituted alcohol **7** in high yield (Scheme 4). The unreacted glycol was separated easily by flash column chromatography. Azidolysis of the monoalcohol gave parasubstituted product **9**, characterized by the AA′XX′ pattern of ^{19}F NMR spectrum. Refluxing P(X)Cl_3 (X = O, S) with **9** in the presence of excess of Et_3N can be monitored via ^{31}P NMR to the disappearance of ^{31}P signals for $PSCl_3$ or $POCl_3$, and 2 equiv of methyl hydrazine was added in situ to obtain the required perfluoroaryl azido-functionalized phosphorus hydrazides **11** and **12**, respectively.

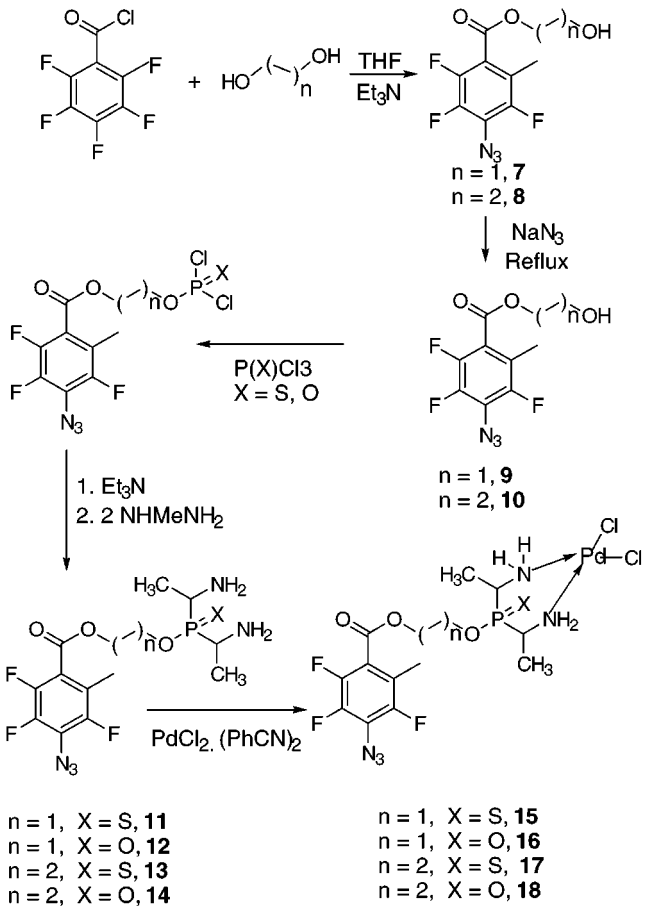
Similarly, the reaction of pentafluorobenzoyl chloride with 1,4-butanediol, followed by azidolysis and treatment with $PSCl_3$ or $POCl_3$ and 2 equiv of methyl hydrazine, produced methylene-bridged perfluoroaryl azidophosphorus hydrazides **13** and **14**. All the ligands were characterized by ^{1}H and ^{31}P NMR, and elemental analysis and ^{1}H NMR showed characteristic doublets around δ 2.8−3.0 due to the phosphorus coupling (^3J (P−H) = 10−12 Hz) with the *N*-methyl protons. The coupling constants values are in the expected range for a number of similar compounds.[23a,24]

Coordination Chemistry. The reaction of the ligands (**5**, **11**−**14**) with $PdCl_2(PhCN)_2$ was performed in CH_2Cl_2

(23) (a) Katti, K. V.; Reddy, V. S.; Singh, P. R. *Chem. Soc.* **1995**, *97* and references therein. (b) Katti, K. V.; Singh, P. R.; Barnes, C. L. *Inorg. Chem.* **1992**, *31*, 4588. (c) Katti, K. V.; Singh, P. R.; Volkert, W. A.; Ketring, A. R.; Katti, K. K. *Appl. Radiat. Isot.* **1992**, *43*, 1151. (d) Katti, K. V.; Ge, Y. W.; Singh, P. R.; Date, S. V.; Barnes, C. L. *Organometallics* **1994**, *13*, 541. (e) Katti, K. V.; Singh, P. R.; Barnes, C. L. *J. Chem. Soc., Dalton Trans.* **1993**, 2153. (f) Singh, P. R.; Jimenez, H.; Katti, K. V.; Volkert, W. A.; Barnes, C. L. *Inorg. Chem.* **1994**, *33*, 736. (g) Wang, M. W.; Volkert, E. W.; Singh, P. R.; Katti, K. K.; Lusiak, P.; Katti, K. V.; Barnes, C. L. *Inorg. Chem.* **1994**, *33*, 1184.

(24) (a) Majoral, J. P.; Kramer, R.; Navech; Mathis, J. *Tetrahedron* **1976**, *32*, 2633 (b) Majoral, J. P.; Caminade. In *Chemistry of Inorganic Ring Systems. Studies in Inorganic Chemistry*; V-14, Ed. Stuedel, Elsevier: Amsterdam, 1992; pp 201. (c) Nicot, B. D.; Mathien, R.; Montauzon de D.; Lavigne, G.; Majoral, J. P. *Inorg. Chem.* **1994**, *33*, 434. (d) Caminamide, A. M.; Majoral, J. P. *Chem. Rev.* **94**, 1183.

Scheme 4



at 25 °C to produce a series of Pd complexes. The interaction of Pd(II) with phosphorus hydrazides can occur in two possible ways, depending on the substitution on phosphorus, although four coordination modes are possible for other transition metals (for example, Cu, Co).[23a] In general, the alkoxy group on phosphorus makes the sulfur or oxygen atom less nucleophilic and hence leads to the formation of a six-membered ring through N−N coordination.[23a] However, the carbon substituents on phosphorus (e.g. $RP(X)(NMeNH_2)$, R = Me, Et, etc; and X = S, O) are known to involve sulfur or oxygen coordination with metals leading to a five-membered ring.[21b] ^{1}H NMR of the complexes (**15**−**18**) revealed only one doublet around δ 3.7−3.2 assigned to the NMe protons, which indicates that the 2 methyl protons flanking phosphorus have similar environments. This could be rationalized in terms of N−N coordination with Pd, which affects NMe protons equally and is confirmed by several X-ray crystal structures reported on similar systems.[23a] In all the compounds the intense absorption band around 2100−2150 cm^{-1} in the IR spectrum indicates the photoactivable group is intact during the chemical manipulations and is available for photoreaction and bioconjugation with proteins and antibodies. The ^{31}P NMR of the complexes exhibit moderate downfield shifts compared with the ligands and exhibit a sharp single resonance indicating the formation of a single species. The IR stretching frequency of P═S for complexes did not show any significant shift compared with the free ligands, indicating that sulfur is not involved in the coordination with Pd. The C, H, and N analyses of all the complexes reveal that they have one ligand per metal center. Irradiation of ligands or complexes did not affect the chelating backbone, as shown by the lack of any appreciable change in the phosphorus chemical shift of these compounds after photolysis.

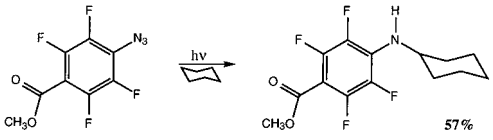
Table 1. Percent Yields of CH Insertion Adducts Obtained by Photolysis of Functionalized Perfluoroaryl Azides in Cyclohexane, and the Retention Times (in Minutes of the CH Insertion Adducts on HPLC[a]

compound	CH insertion yield[a] (%)	retention time (R_t) (min)
1, **2**, **4**	<5	
5	73	2.8
6	70 ± 5	3.3
11	90	3.6
12	93	3.8
13	92	4.7
14	89	4.5
16	75 ± 5	3.2

[a] The solvent was CH_3CN and H_2O in a ratio of 2:1 (flow rate, 1 mL/min). [b] Measured by the integration of fluorine signals which agrees well with the values obtained from chromatographic separation of photoproducts using hexane:ethyl acetate:methanol in 9:9:1 ratio on silica gel column. For NMR, a 2.5 × 10^{-3} M solution of azide in cyclohexane was photolyzed in NMR tube for 1−2 h at room temperature at >320 nm.

Photochemical Insertion. Inspection of Table 1 reveals that photolysis of tetrafluorinated aryl azides containing a parahydrazone substituent in cyclohexane fails to produce C−H insertion adducts. In each case, the major products formed upon photolysis of **1**, **2**, and **4** were the corresponding anilines. Aniline-type reduction products are usually associated with reactions of triplet aryl nitrenes. This was confirmed by the finding that triplet-sensitized photolysis of **1**, **2**, and **4**, or direct photolysis in methanol (a solvent that catalyzes intersystem crossing of singlet nitrene to their triplet ground states[13]) also forms the same aniline products.

Soundarajan and Platz[25] reported that photolysis of **19** in cyclohexane leads to the formation of an adduct in 57% yield, in contrast to our work with tetrafluorohydrazone azides **1**, **2**, and **4**.



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We find that this is a general result. Compounds **5** and **11**−**16** are tetrafluorinated but have carboxamide or ester functionality para to the azide substituent, separated by methylene groups. Photolysis of these azides in cyclohexane produces CH insertion adducts in excellent yield (Table 1, Scheme 5).

The placement of an ester or amide group with a bridge of methylene groups between the photolabel and the chelating moiety improves upon our previously reported results with similar compounds.[21] For example, photolysis of **11**−**14** in neat cyclohexane gives CH insertion products (**11a**−**14a**, Scheme 5) in almost quantitative yield, whereas the Pd complex **16** shows an insertion yield 75 ± 5% compared with the Pd complex (45%) without the methylene bridge.[21] These are the most efficient aryl nitrene C−H insertion reactions known with cyclohexane.

(25) Soundarajan, M.; Platz, M. S. *J. Org. Chem.*, **1990**, *55*, 2034.

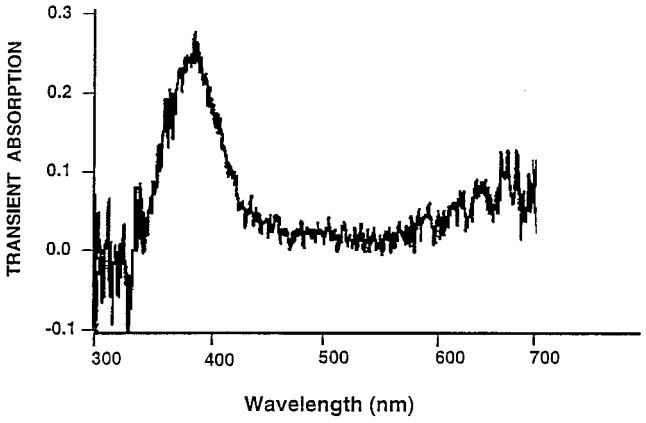
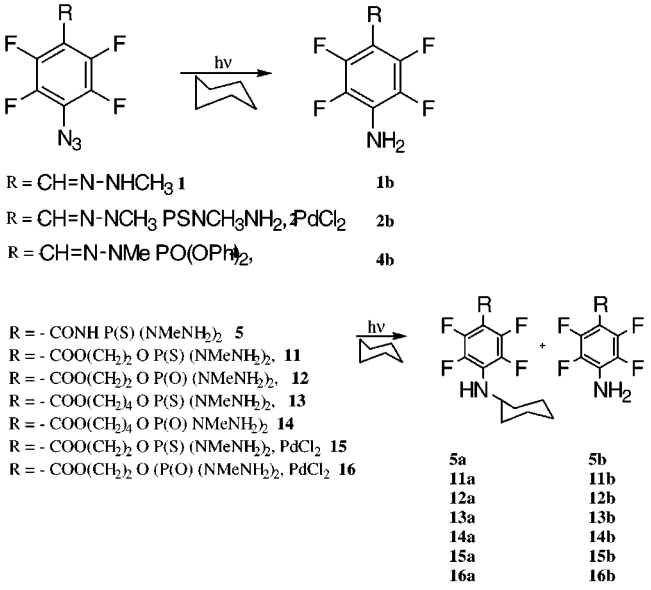
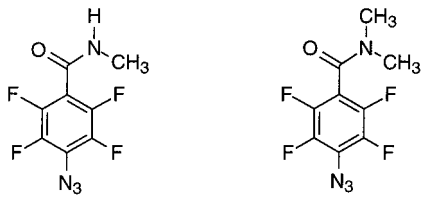


Figure 1. The transient absorption spectrum produced upon LFP of **1** in CH_3CN. The transient absorption spectrum of ketenimine **1c** and triplet nitrene **1e** was recorded 1.4 μs after the laser pulse over a 200-ns window at ambient temperature.

Scheme 5



Laser Flash Photolysis Studies. Tetrafluorinated azide ester **19** and its amide analogues **20** and **21** have been studied by laser flash photolysis (LFP) techniques.[26a] The singlet nitrenes derived from these precursors were remarkably long-lived. Nitrenes derived from **1**, **2**, and **4** were also studied by LFP methodology to understand their failure to give cyclohexane adducts.



20 **21**

LFP (XeCl excimer, 308 nm, 17 ns) of azide **1** in acetonitrile at room temperature produces a transient spectrum with a broad absorption maximum around 380 nm (Figure 1) and weak broad bands at 480 and 650 nm. Upon LFP of **1** in acetonitrile containing 1 M diethylamine, a known quencher of didehydroazepines, very weak transient absorption was observed at 380, 450, and 650 nm. Thus, by analogy with the photochemistry of other polyfluorinated aryl azides, the strongly absorbing transient of Figure 1 is attributed to ketenimine **1c**,[13,16−17] and the weak bands at 380, 450, and 650 nm are associated with triplet nitrene **1e**. A weak transient spectrum of triplet nitrene **1e** was also detected in CH_2Cl_2 at −32 °C and in methanol.

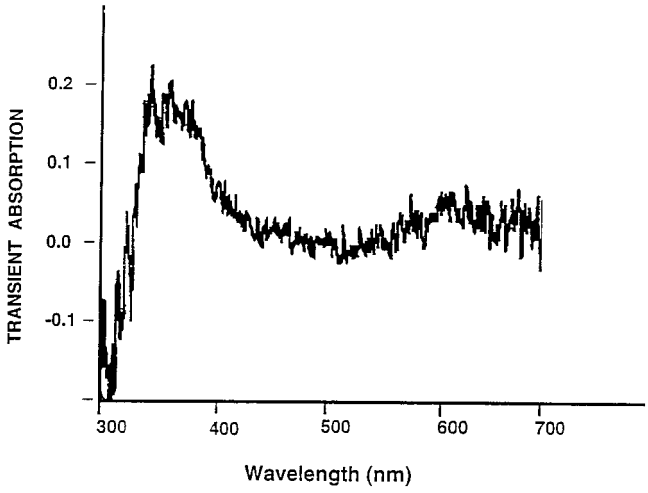
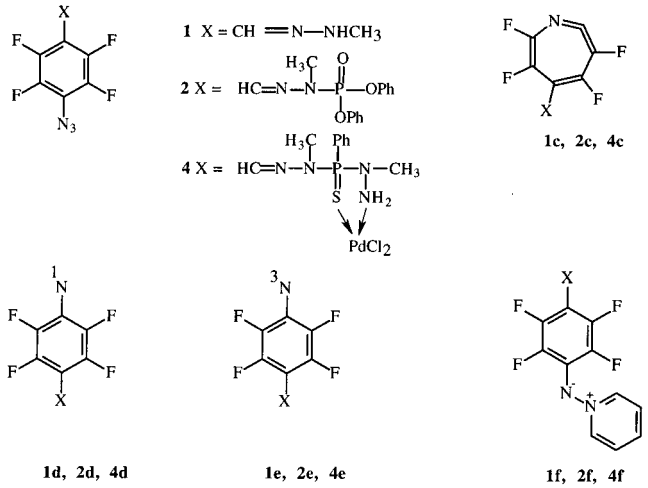


Figure 2. The transient absorption spectrum produced upon LFP of **4** in CH_3CN. The transient absorption spectrum of ketenimine **4c** and triplet nitrene **4e** was recorded 1.4 μs after the laser pulse over a 200-ns window at ambient temperature.



LFP of **4**, in acetonitrile in the absence of pyridine, produced a transient spectrum (Figure 2) that contains maxima at 345, 370, and 620 nm. The bands at 345 and 370 nm must be associated with different species because they have different lifetimes (41 and 32 μs, respectively). The two species observed in acetonitrile are attributed to ketenimine **4c** (370 nm) and triplet nitrene **4e** (345 and 620 nm). Only triplet nitrene **4e** is observed in acetonitrile containing diethylamine (where **4c** is trapped), in methanol solvent [which is known to accelerate intersystem crossing (ISC[13])], and in CH_2Cl_2 at low temperature where ISC is favored relative to the rearrangement of singlet nitrenes[13] (Supporting Information, Figures 1−3, respectively). The positions of these bands

(26) (a) Marcinek, A.; Platz, M. S.; Chan, S. Y.; Floresca, R.; Rajagopalan, K.; Golinski, M.; Watt, D. *J. Phys. Chem.* **1994**, *98*, 412. (b) Marcinek, A.; Platz, M. S. *J. Phys. Chem.* **1993**, *97*, 12674.

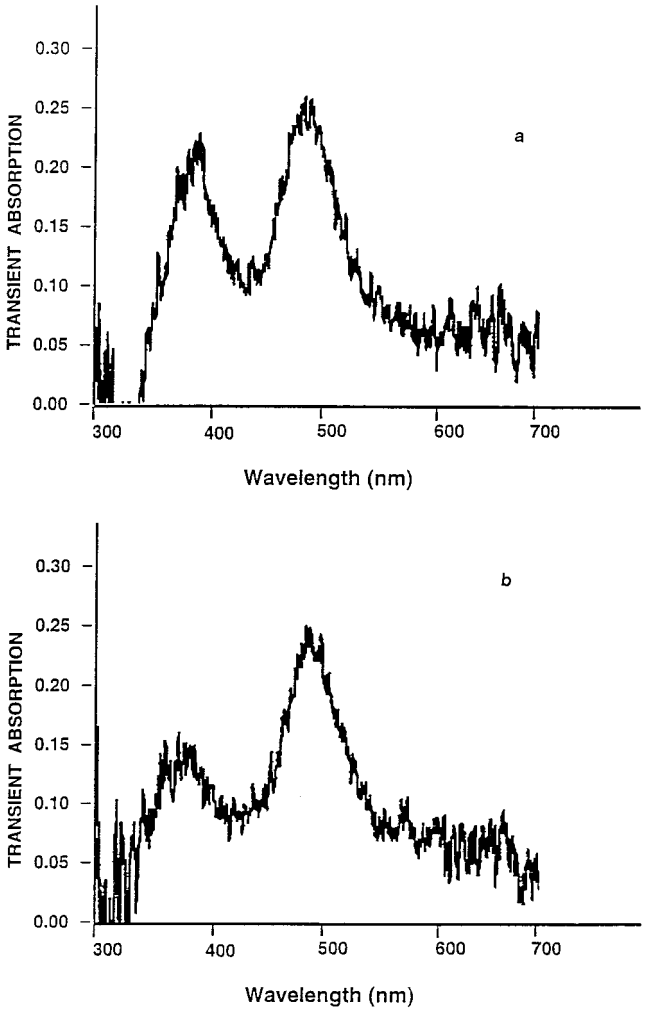


Figure 3. (a) The transient absorption spectrum produced upon LFP of **1** in CH_3CN containing pyridine. The spectrum of ketenimine **1c** and nitrene-pyridine ylide **1f** was recorded 10 μs after the laser pulse over a 200-ns window, at ambient temperature. (b) The transient absorption spectrum produced upon LFP of **4** in CH_3CN containing pyridine. The transient absorption spectrum of ketenimine **4c** and nitrene-pyridine ylide **4f** were recorded 1.4 μs after the laser pulse over a 200-ns window at ambient temperature.

are not unreasonable because pentafluoroketenimine has a broad absorption between 380 and 400 nm[13d] and pentafluoro triplet phenyl nitrene absorbs sharply at 320 nm, more broadly at 370 nm, and has a weak band in the visible region of the spectrum extending to 550 nm.[13a] Similarly, LFP of **2** in acetonitrile produces transient absorption at 360, 570, and 610 nm. The same bands are obtained upon LFP of **2** in methanol, a solvent that catalyzes ISC. These transient spectra are associated with triplet nitrene **2e**.

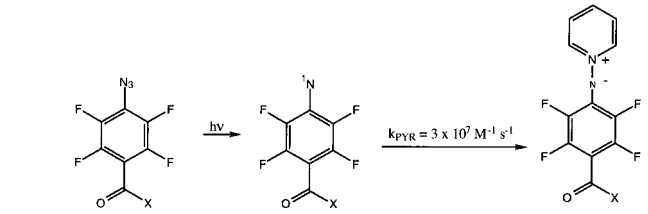
LFP of **1**, **2**, or **4** in acetonitrile containing pyridine produces transients that absorb strongly around 500 nm (Figure 3). Based on analogy with our previous work with polyfluorinated azides,[13,26] these transients are attributed to nitrene-pyridine ylides **1f**, **2f**, and **4f**, respectively.

Discussion

The correlation of lifetimes of singlet nitrenes derived from azides with CH insertion yields in organic solvents, studied by chemical trapping and flash photolysis techniques, results in the development of more efficient photolabeling agents for cross-linking biomolecules. For example, Marcinek et al.[26] have studied azides **19**, **20**, and **21** by LFP techniques and found that the corresponding singlet nitrenes react with pyridine with absolute rate constant $k_{PYR} = 3 \times 10^7$ M^{-1} s^{-1} at ambient temperature.

Table 2. Lifetimes of Functionalized Perfluoroaryl Nitrenes in Different Solvents at Ambient Temperature

X	*t* (ns)	solvent
$CH=NNHCH_3$	5	CH_3CN
$CH=NNCH_3PSNCH_3NH_2 \cdot PdCl_2$	4	CH_3CN
COX (X = OCH_3, $NHCH_3$, $N(CH_3)_2$)	208[26]	CH_2Cl_2
F[27]	38	CH_2Cl_2
C_6F_5[27]	259	n-C_5H_{12}
C_6F_5[27]	254	CH_2Cl_2
C_6F_5[27]	220	CH_3CN
C_6F_5[27]	65	CH_3OH



19	x = OCH_3	**19d**	**19f**
20	x = $NHCH_3$	**20d**	**20f**
21	x = $N(CH_3)_2$	**21d**	**21f**

The absolute values of k_{PYR} of singlet pentafluorophenyl nitrene and perfluorobiphenyl nitrene are 6×10^7 M^{-1} s^{-1} at ambient temperature.[27] It was not possible to measure the lifetime of singlet nitrenes **1d** and **4d** directly. Thus, we measured the optical yields (A_y) of ylides **1f** and **4f** produced per laser pulse as a function of pyridine concentration (Figure 4). Plots of $1/A_y$ versus 1/[pyridine] are linear (Figure 5). The ratio of the intercept/slope of such plots is $k_{PYR}\,\tau$, where k_{PYR} is the absolute rate constant of reaction of singlet nitrene with pyridine, and τ is the lifetime of singlet nitrene in the absence of pyridine.[13,26,27] However, if we assume that these nitrenes react with pyridine with $k_{PYR} = 3 \times 10^7$ M^{-1} s^{-1}, we deduce that their lifetimes are 5 and 4 ns, respectively, in acetonitrile.

Singlet aryl nitrenes decay by rearrangement, intersystem crossing (k_{ISC}), and reaction with solvent (k_{RH}[RH], see Scheme 1).[16,26] The lifetime τ in the absence of pyridine is simply related to the absolute rate constants of Scheme 1, by eq 1.[26,27]

$$1/\tau = k_R + k_{ISC} + k_{RH}[RH] \qquad (1)$$

Values of the lifetime of various polyfluorinated singlet arylnitrenes are given in Table 2. It is clear that hydrazine singlet nitrenes **1d** and **4d** have lifetimes that are 40−50 times shorter than those of ester and amide nitrenes **19d** and **21d**.

For a singlet nitrene to be useful as an highly efficient photolabel, eq 2 must hold

(27) Gritsan, N. P.; Zhai, H. B.; Yuzawa, T.; Karwick, D.; Brooke, J.; Platz, M. S. *J. Phys. Chem.* **1997**, *101*, 2833.

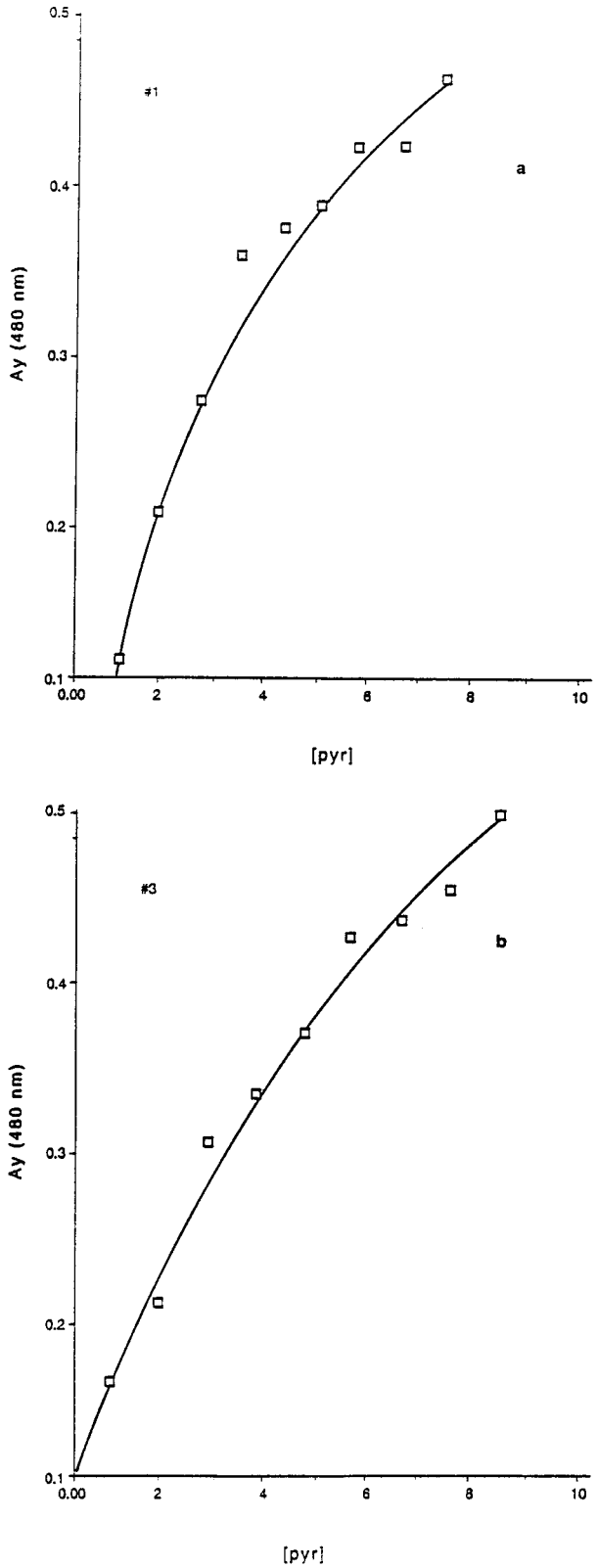


Figure 4. (a) The yield of ylide (A_y) formed upon LFP of **1** in CH_3CN as a function of pyridine concentration. (b) The yield of ylide (A_y) formed upon LFP of **4** in CH_3CN as a function of pyridine concentration.

$$k_{RH}[RH] \geq k_R + k_{ISC} \quad (2)$$

Marcinek et al.[26] showed that for nitrenes **19d** − **21d** that $k_R + k_{ISC} = 4.8 \pm 0.5 \times 10^6\ s^{-1}$ at ambient temperature. Intersystem crossing rates are not temperature-dependent, and k_{ISC} was found to be $3.0-3.3 \times 10^6\ s^{-1}$ for nitrenes **19d** −**21d**.[26] This allowed Marcinek et al. to deduce that $k_R = 1.5-1.8 \times 10^6\ s^{-1}$ at ambient temperature with Arrhenius barriers of $A = 1.2 \times 10^{13}\ s^{-1}$ and $E_a = 9$ kcal/mol for tetrafluorinated singlet nitrene ester and amides.

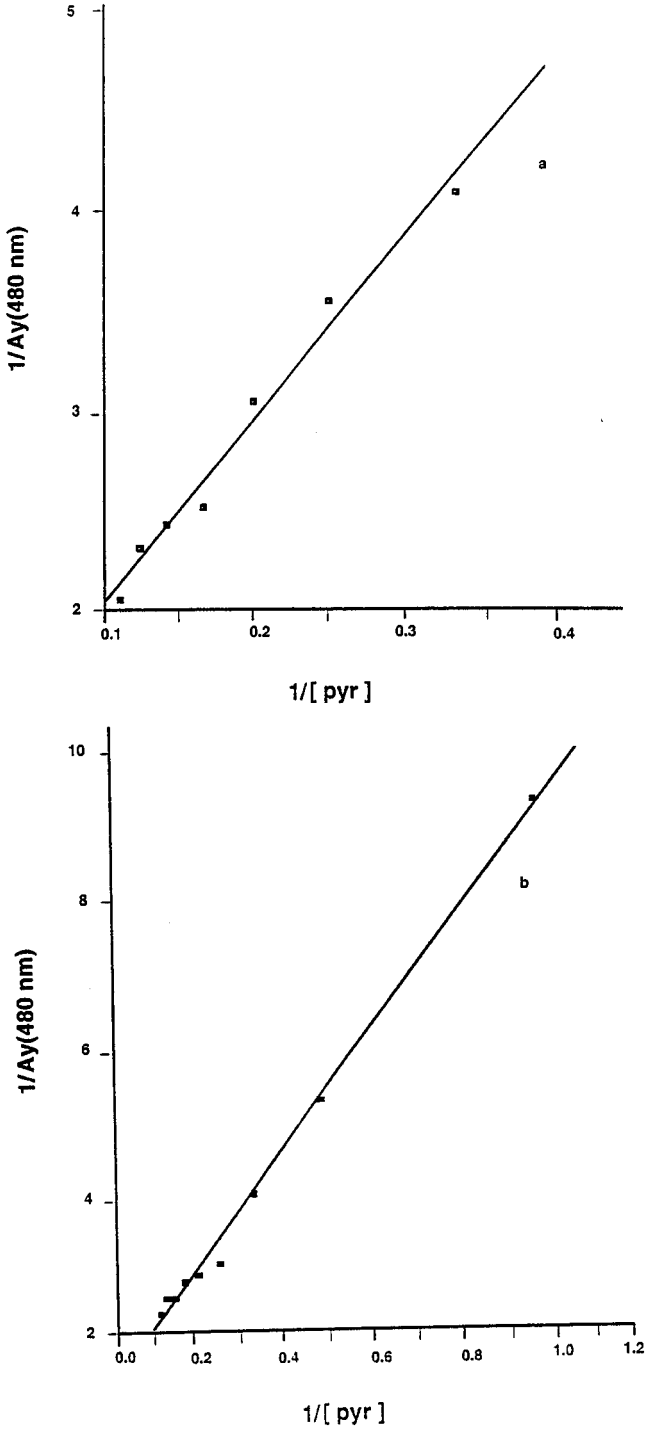
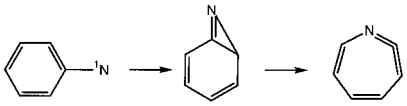


Figure 5. (a) A double-reciprocal treatment of the data of Figure 4a. (b) A double-reciprocal treatment of the data of Figure 4b.

Karney and Borden[28] calculated the rearrangement of singlet arylnitrenes to ketenimines and found that it is a two-step process.

(28) (a) Karney, W. L.; Borden, W. T. *J. Am. Chem. Soc.* **1997**, *119*, 1378. (b) Karney, W. L.; Borden, W. T. *J. Am. Chem. Soc.* **1997**, *119*, 3347.



The first step is the slow step. It has a calculated barrier of ~6 kcal/mol, in agreement with the measured value of rearrangement of parent singlet phenylnitrene.[29]

Karney and Borden[28] found that ortho methyl and ortho fluoro substituents raise the barrier to rearrangement by 2−3 kcal/mol by a steric effect in the transition state.[28] The steric effects in **1d** and **2d** appear to be no different from those in singlet nitrenes **19d**−**21d**. Thus, we expect that k_R will be comparable throughout this series. Fast rearrangement of singlet nitrenes **1d** and **2d** probably is not responsible for the short lifetimes of these species relative to **19d**−**21d**.

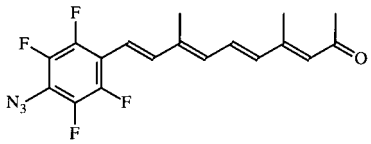
Photolysis of **1** and **2** gives the corresponding anilines almost exclusively in good yield (Scheme 5). This indicates that k_{ISC} of **1d** and **2d** is larger than in **19d**−**21d**.

Unlike singlet phenylcarbene,[29] singlet phenylnitrene[30] has an open-shell electronic configuration.[31] Thus, singlet nitrenes undergo concerted bond insertion processes more slowly than do carbenes.[28,32] Substitution patterns which make closed-shell configurations more accessible will accelerate concerted reactions, which lead to productive photolabeling.

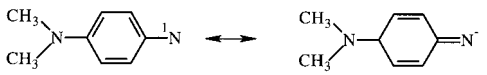
Spin−orbit coupling (SOC)-ISC is allowed in carbenes where a closed-shell "ionic" configuration is coupled to an open-shell "covalent" configuration.[33] SOC−ISC is forbidden between open-shell singlet and triplet states as in a nitrene.[32,33] This explains why ISC is 10^{3-4} times faster in carbenes[34] than in nitrenes.[30]

In the Schiff base, the parahydrazone group can conjugate with a singlet nitrene as shown below.

This conjugation stabilizes a closed-shell singlet configuration and lowers the singlet−triplet gap, both factors which accelerate ISC. Hence, we believe that k_{ISC} in **1d** and **2d** exceeds that of **19d**−**21d**. This shortens the lifetimes of **1d** and **2d** and reduces the efficiency of CH insertion of these species ($k_{ISC} \gg k_{RH}$[RH], Scheme 1) and reduces their utility as photolabeling reagents. Similarly, orthosubstitution of perfluoroaryl azides by electron-donating *N*-methyl amine group[21e] or phosphinimine group[21f] has been shown to reduce CH insertion yield compared with their precursor perfluoroaryl benzonitrile.[21d] The iminophosphorano moiety (N=P) attached to the perfluoroaryl ring participates in the extended conjugation, thereby releasing electron density into the empty orbital of the singlet nitrene compensating for the electron-withdrawing capacity of the parasubstituted nitrile group. Similarly, electron release from the Pd metal through back-bonding to iminophosphorano nitrogen enhances the electron flow into the empty orbital of singlet reducing the electrophilicity of nitrene. These conclusions agree well with the results on the *trans*-(4-azido-2,3,5,6-tetrafluorophenyl)-3,7-dimethyl-2,4,6,8-nonatetranal)



which failed to yield any significant CH insertion in cyclohexane.[35] The addition of the extended polyene side chain, which is in conjugation with the azido group, apparently modifies the reactivity of the singlet nitrene making it less likely to insert into either the CH or the NH bonds of model solvents. Failure of this compound to insert even in the seemingly favorable protein matrix conditions confirms our earlier suggestion that there is a need for the development of intermolecular CH/NH insertion chemistry in model solvents which has a close relationship with the success of perfluoroaryl azides as photolabels on specific biomolecules. Our results are also consistent with the work of Miura and Kobayashi[36] with nitrene **22d**. The absolute rate constant of ISC in **22d** is $8.3 \pm 0.2 \times 10^9$ s^{-1}, which is 3 orders of magnitude larger than k_{ISC} in parent singlet phenyl nitrene.[30]



22d

Photolabeling of Human Serum Albumin. The efficiency of covalent modification of biomolecules by photolabeling techniques depends on the optimization of productive versus nonproductive avenues for perfluoroaryl nitrenes which is controlled by (a) electronic tuning of perfluoroaryl azides para to azido group, (b) net electrophilicity of singlet nitrene through steric factors especially with electron-rich metals, and (c) the vicinity of the biomolecule around which nitrene is generated. The three types of synthetic designs for obtaining BFPCAs provide an opportunity to study the electronic as well as the steric effects of metalated systems on the photochemistry of perfluoroaryl azides. Although, BFPCAs showed high CH insertion in model organic solvents, the intriguing question is whether the long lifetime of singlet nitrenes derived from amides and esters can be extended for insertion into biomolecules under buffer conditions and at tracer level concentrations. One of our long-term objective is to demonstrate the feasibility of formation of instant ^{99m}Tc-labeled antibody fragment (Fab) conjugation kits using photolabeling agents to target Fab to specific sites of tumor. Use of HSA for attaching BFPCAs constitute a good model for antibody fragments because

(29) (a) Matzinger, S.; Bally, T.; Patterson, E. V.; McMahon, R. J. *J. Am. Chem. Soc.* **1996**, *118*, 1535. (b) Schreiner, P. R.; Karney, W. L.; Schleyer, P. V. R.; Borden, W. T.; Hamilton T. P.; Schaefer, H. F., III. *J. Org. Chem.* **1996**, *61*, 7030 (c) Wong, M. W.; Wentrup, C. J. *J. Org. Chem.* **1996**, *61*, 7022.

(30) Gritsan, N. P.; Yuzawa, T.; Platz, M. S. *J. Am. Chem. Soc.* **1997**, *119*, 5059.

(31) (a) Kim, S. J.; Hamilton, T. P.; Schaefer, H. F., III *J. Am. Chem. Soc.* **1992**, *114*, 5349. (b) Hrovat, D. A.; Waali, E. E.; Borden, W. T. *J. Am. Chem. Soc.* **1992**, *114*, 8698. (c) Travers M. J.; Coules, D. C.; Clifford, E. P.; Ellison, G. B. *J. Am. Chem. Soc.* **1992**, *114*, 8699.

(32) Platz, M. S. *Acc. Chem. Res.* **1995**, *28*, 487.

(33) Salem, L.; Rowland, C. *Angew. Chem., Int. Ed. Engl.* **1972**, *11*, 92.

(34) (a) Sitzmann, E. V.; Langan, J.; Eisenthal, K. B. *J. Am. Chem. Soc.* **1984**, *106*, 1868. (b) Grasse, P. B.; Brauer, B.-E.; Zupancic, J. J.; Kaufmann, K. J.; Schuster, G. B. *J. Am. Chem. Soc.* **1983**, *105*, 6833.

(35) Beischel, C. J.; Knapp, D. R.; Govindji, R.; Ebrey, T. G.; Crouch, R. K. *Photochem. Photobiol.* **1994**, *60*, 64.

(36) Miura, A.; Kobayashi, T. *J. Photochem. Photobiol A.* **1990**, *53*, 223.

Table 3. Percentage of Covalent Attachment of ^{99m}Tc Complex with HSA

conditions	% of activity at 8.0-cm spot	% of activity at 13.2-cm spot	% of activity at 14.5-cm spot
HSA + ^{99m}Tc complex (before photolysis)	6.63 ± 2	86.14 ± 3	
HSA + ^{99m}Tc complex (10 min of photolysis)	73.13 ± 2	12.74 ± 3	13.26 ± 2
HSA + ^{99m}Tc complex (15 min of photolysis)	79.98 ± 2	6.04 ± 3	15.83 ± 2
^{99m}Tc complex in buffer	13.5 ± 2		85.8 ± 2

[a] A 2 x 10−6 M solution of 99mTc complex was photolyzed (320 nm) for 10 and 15 min at room temperature. The yield of covalently attached photoprobe to HSA was measured by the integration of the peaks of radiochemical scans (bioscan) subtracted by the residual radioactivity before photolysis (control experiment).

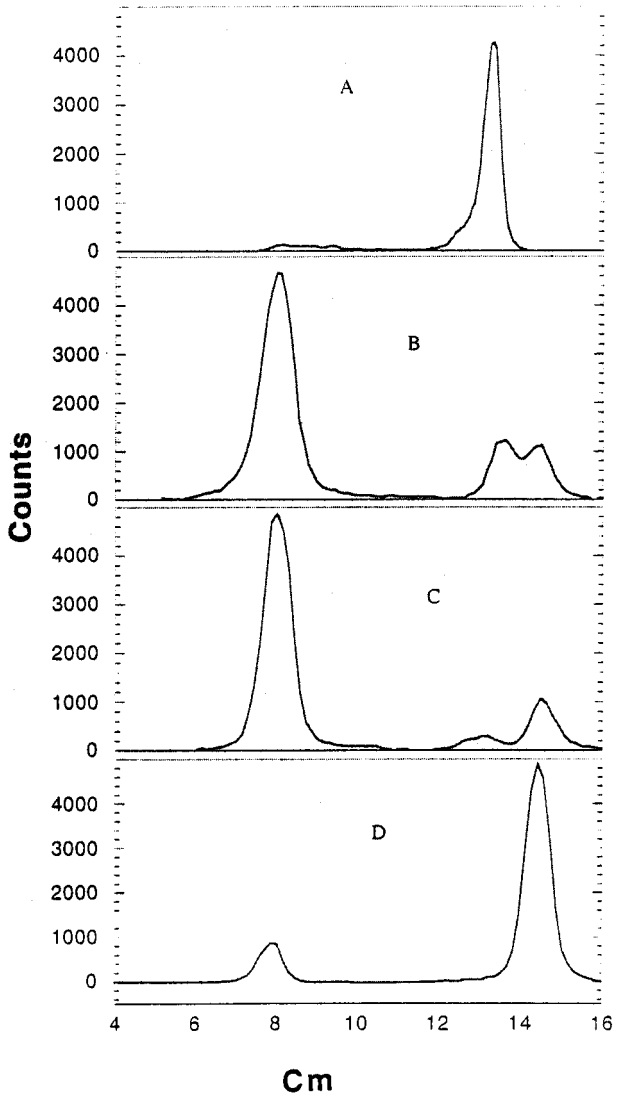


Figure 6. (A) TLC of ^{99m}Tc complex **12** + HSA before photolysis in acetone, (B) 10 min of photolysis, (C) 15 min of photolysis, and (D) photolysis without HSA in phosphate-buffered saline.

HSA has been used for hepatic imaging.[7g] In this connection, we characterized ^{99m}Tc complex of **12** using paper chromatography (PC), thin-layer chromatography (TLC), electrophoresis, high-performance liquid chromatography (HPLC) and solvent extraction procedures (see Supporting Information, Figures 5−7). The ^{99m}Tc complex was incubated with HSA for an hour before photolysis. The elution profile of the unphotolyzed mixture in TLC showed a major radiochemical spot at 13.2 cm (origin of spot at 8.0 cm) corresponding to the radiochemical complex (Figure 6A) indicating that the photolabile complex can be separated from HSA before photolysis (control experiment). Sequential photolysis of the mixture shows a considerable shift of radioactivity to the origin of spot (8.0 cm, Figure 6B and C after 10 and 15 min, respectively) indicating that the complex is associated with HSA which cannot move in TLC. Previously, we showed such an association to be covalent in nature for similar complexes.[21e,22] A new spot at 14.5 cm also appears after photolysis, which coincides with the retention time of the photolytic product in the absence of HSA (buffer, Figure 6D). This spot can be assigned to a triplet nitrene-derived aniline product based on previous observations.[21,22] The low fraction of radioactivity seen at the origin in Figure 6D may be caused by the partial oxidation of the complex to $^{99m}TcO_2$, which is not mobile in TLC when acetone is used as eluent. However, in the control experiment (Figure 6A), very little oxidation product is seen (6%). Based on the percentage of the radioactivity at the origin with respect to the total activity, we can reasonably assume the efficiency of covalent attachment of the probe to be approximately 72 ± 5% (Table 3). These results demonstrate that the correlation of long lifetimes of singlet nitrenes with intermolecular insertion efficiency in organic solvents can be extended successfully to the covalent attachment of biomolecules at tracer level concentrations. The complete characterization of the radiochemical complex and the quantification of the efficiency of covalent attachment of ^{99m}Tc complex with HSA using HPLC (dual detectors, UV at 254 nm and a radiochemical detector) is described in the Supporting Information which will be reported elsewhere.

Conclusions

In view of the increasing need for the development of highly efficient CH insertion molecular probes for covalent attachment to receptors and antibodies in biochemistry and in nuclear medicine, we synthesized several BFPCAs. This series of compounds exhibit a favorable intermolecular insertion chemistry, for example, the largest CH insertion yield of a nitrene ever reported with cyclohexane (compounds **11**−**14** and **16**). LFP studies of BFPCAs establish that the singlet nitrene lifetimes of hydrazone derivatives are an order of magnitude shorter than those observed for perfluoroaryl nitrenes with ester or amide groups in the para position. The longer lifetimes correlate well with the higher yields of CH insertion observed in model solvents with the ester- and amide-substituted compounds. Moderate to high efficiency of photochemical attachment of ^{99m}Tc-labeled BFPCA to HSA demonstrates that the observations in model solvents can be extended to tracer levels in buffer conditions. The predictable photochemistry of this class of highly electrophilic nitrenes establishes a firm basis for the attachment of radiometalated probes to biomol-

ecules of interest. In this connection, studies on the identification of specific receptor-based molecular target systems are in progress.[37]

Experimental Section

All synthetic procedures were conducted in a dry nitrogen atmosphere using standard Schlenk tube techniques and prepurified solvents. Reactions involving the synthesis of azides were carried out under subdued light by wrapping the flasks with aluminum foil. Diphenoxyphosphoryl methyl hydrazine **3** was prepared as reported previously.[23g] NMR spectra were recorded in $CDCl_3$ on a 250-MHz spectrometer, and chemical shifts are reported in ppm downfield from $SiMe_4$ for ^1H NMR. The ^{31}P NMR chemical shifts are reported with respect to 85% H_3PO_4 as an external standard, and positive shifts lie downfield from the standard. ^{19}F NMR chemical shifts are reported with respect to trifluorotoluene as an external standard. Infrared spectra were recorded as KBr pellets on a Fourier transform-IR spectrophotometer. Elemental analysis for the new compounds were performed by Oneida Research Services, Inc., New York. UV-visible spectra were recorded either in cyclohexane or in acetonitrile on a Hewlett-Packard diode array spectrophotometer.

Photolyses were carried out with a 200-W high-pressure Hg lamp directed through water as a filter. The total lamp output was measured by ferrioxalate actinometry to be 2 mE/h in the range of 320−360 nm. The photolysis solution absorbs only a small fraction of the incident light at the red edge of the photoprobe absorption. Approximately 50% of the focused beam was intercepted with a 1-cm^2 quartz cuvette for most experiments. For specific in situ NMR experiments, the solution in the NMR tube was irradiated directly, intercepting 10% of the incident light. Irradiation of solutions was continued to the destruction of azide, monitored by C-18 reverse-phase HPLC. Retention time (R_t) values for cyclohexane adducts are reported in minutes using eluent CH_3CN:H_2O (2:1). Solutions (10^{-5} M) were purged with prepurified nitrogen for 5 min before photolysis. Solutions of higher concentrations (10^{-3}−10^{-2} M) were photolyzed (2−3 h) for chromatographic analysis of the photoproducts. The experimental apparatus for conducting LFP studies has been described elsewhere.[13h] For bioconjugation experiments, 1 mg/mL of the ligand **12** (2.319 μmol) is incubated with HSA in a glass tube for 10−15 min (69 mg in 10 mL phosphate-buffered saline, i.e. 2 μmol, approximately, in 1:1 ratio).

Synthesis of 1 and 4. Dropwise addition of methyl hydrazine dissolved in absolute ethyl alcohol (0.460 g, 9.9 mmol) into a solution of perfluoroazidobenzaldehyde (2.19 g, 9.9 mmol) also taken in alcohol at 0 °C was followed by stirring for 30 min. The solution was filtered and evaporated to yield a brown precipitate that was redissolved in methanol and evaporated slowly to obtain crystalline material **1** (89% yield). ^1H NMR ($CDCl_3$) δ, 2.91 (s, 3H), 3.2 (b, 1H), 7.6 (s, 1H), ^{19}F NMR ($CDCl_3$) δ, −84.4 (m, 2F), −92.8 (m, 2F). Anal. Calcd for $C_8H_5N_5F_4$; C, 38.88; H, 2.04; N, 28.34. Found: C, 38.85; H, 2.10; N 28.51.

A solution of diphenoxyphosphoryl methyl hydrazine **3** reported earlier[23g] in absolute ethyl alcohol (2.78 g, 9.9 mmol) was added dropwise into a solution of perfluoro azido benzaldehyde (2.19 g, 9.9 mmol) also taken in alcohol at 0 °C. The mixture was stirred for 30 min, and the reaction was monitored by ^{31}P NMR spectroscopy. The solution was filtered and the precipitate was washed with ethanol and dissolved in CH_2Cl_2 and evaporated slowly to obtain crystalline material **4** (89% yield). ^1H NMR ($CDCl_3$) δ, 3.21 (d, ^3J = 11.2 Hz. 3H), 7.0−7.5 (m, 10H), 7.6(s, 1H), ^{31}P NMR δ, −5.3, ^{19}F NMR ($CDCl_3$) δ, −75.1 (m, 2F), −85.3 (m, 2F), Anal. Calcd for $C_{20}H_{14}N_5O_3PF_4$; C, 50.12; H, 2.94; N, 14.61. Found: C, 50.22; H, 3.01; N, 14.53.

Similarly, the palladium complex **2** (Scheme 1) with bis hydrazine phosphorus sulfide (BHP) was prepared as reported earlier[22b] and used as such in flash photolysis studies.

(37) Pandurangi, R. S.; Rao, S. N. (unpublished results).

Synthesis of Triplet Product of 1 via Photolysis. A solution of **1** (650 mg, mmol) in a mixture of CH_3OH and H_2O (9:1) was photolyzed for 5 min in a quartz tube. The photochemical reaction was monitored to the level of 95% disappearance of the ^{19}F signals of the starting material. The solution was filtered and evaporated and redissolved in CH_3CN and cooled to yield crystalline material **1b** (87% yield, Scheme 4). ^1H NMR ($CDCl_3$) δ, 7.5 (s, 1H), 4.2 (s, 2H), 3.2 (b, 1H), 2.86 (s, 3H). ^{19}F NMR ($CDCl_3$) δ, −84.8 (m, 2F), −101.6 (m, 2F). Anal. Calcd for $C_8H_7N_3F_4$; C, 43.43; H, 3.19; N, 34.38. Found: C, 43.78; H, 3.10; N, 34.51.

Synthesis of Triplet Product of 4 via Photolysis. A solution of **4** (500 mg, 1.04 mmol) in a mixture of CH_3OH and H_2O (9:1) was photolyzed for 5 min in a quartz tube. The photochemical reaction was monitored to the level of 95% disappearance of the ^{19}F signals of the starting material. The solution was filtered and evaporated and redissolved in CH_3CN and cooled to yield crystalline material **4b** (92% yield, Scheme). ^1H NMR ($CDCl_3$) δ, 2.92 (d, ^3J = 11.4 Hz, 3H), 4.2 (b, s, 2H), 7.0−7.5 (m, 10H), 7.63(s, 1H), ^{31}P NMR, δ, −5.8, ^{19}F NMR ($CDCl_3$) δ, −83.9 (m, 2F), −101.7 (m, 2F). Anal. Calcd for $C_{20}H_{16}N_3O_3PF_4$, C, 52.98; H, 3.55; N, 9.27. Found: C, 52.61; H, 3.60; N, 9.22.

Synthesis of 5 and 6. A solution of 4-azido-2,3,5,6-tetrafluorobenzamide prepared as reported earlier[13a] (1 g, 4.27 mmol), taken in dry CH_3CN, was slowly added dropwise to a solution of $PSCl_3$ (2.169 g, 12.81 mmol) at room temperature in the presence of excess triethylamine. The solution was refluxed for 8 h and monitored by ^{19}F NMR until the fluorine signals due to the starting material disappear. The excess $PSCl_3$ was removed under high vacuum, the resulting oil was dissolved in $CHCl_3$, and 2 mol of methyl hydrazine in $CHCl_3$ (0.543 g, 11.78 mmol) was slowly added and stirred for an hour. The solution was filtered and the filtrate was evaporated in a vacuum to yield brown solid **5**. The solid was dissolved in hot CH_3CN and cooled at −5° C to obtain an analytically pure sample (67% yield). ^1H NMR ($CDCl_3$), δ, 4.8 (b, 1H), 2.97 (d, J(P−H) = 11.2 Hz, 6H), 3.3 (b, 4H). ^{31}P NMR δ, 67.8, ^{19}F NMR ($CDCl_3$) δ, −75.3 (m, 2F), −87.4 (m, 2F). Anal. Calcd for $C_9H_{11}N_8F_4OPS$; C, 27.99; H, 2.87; N, 29.01. Found: C, 27.71; H, 2.75; N, 29.09.

Synthesis of 11−14. To 245 mg (3.95 mmol) of ethylene glycol in dry THF was added 1 g (3.95 mmol) of pentafluorobenzoyl chloride, also in THF. TLC of the mixture showed 2 spots presumably caused by the bis- and monosubstitution products. The mixture was separated chromatographically on a silica gel column using hexane, ethyl acetate and methanol in a 9:3:1 ratio to produce a colorless oil **7** (87% yield). ^1H NMR ($CDCl_3$) δ, 4.53 (t, ^2J = 4.6 Hz, 2H), 3.97 (t, ^2J = 4.7 Hz, 2H), 1.42 (b, 1H). ^{19}F NMR ($CDCl_3$) δ, −75.5 (m, 2F), −88.4 (m, 2F), −97.2 (m, 2F). Anal. Calcd for $C_9H_5F_5O_3$; C, 42.21; H, 1.97. Found: C, 42.31; H, 1.90.

A solution of pentafluorobenzoyl chloride (2 mL, 13.88 mmol) in dry THF was added to a solution of 1,4-butanediol in THF (1.25 mL, 14.11 mmol) dropwise and refluxed overnight. The solution was cooled and treated with Na_2CO_3 to neutralize the acid byproduct. The solution mixture was extracted with ether (2 × 10 mL), and the organic phase mixture was separated chromatographically on a silica gel column using hexane, ethyl acetate, and methanol in a 9:3:1 ratio to produce a colorless oil **8** (yield 78%). ^1H NMR ($CDCl_3$) δ, 1.41 (b, 1H), 1.72 (m, 2H), 1.82 (m, 2H), 3.75 (t, ^2J = 6.2 Hz, 2H) 4.41 (t, ^2J = 6.4 Hz, 2H). ^{19}F NMR ($CDCl_3$) δ, −75.8 (m, 2F), −88.7 (m, 2F), −97.6 (m, 2F). Anal. Calcd for $C_{11}H_9O_3F_5$; C, 46.51; H, 3.21. Found: C, 46.63; H, 3.28.

Azidolysis of Alcohol. About 1.1 g of alcohol **7** (4.3 mmol) was taken in acetone and mixed with 300 mg of NaN_3 (4.61 mmol) in acetone/water and refluxed for 8 h. Water was added to the mixture and extracted into ether and dried over $MgSO_4$ to produce yellow liquid **9** (yield, 79%). ^1H NMR ($CDCl_3$) δ, 4.63 (t, ^2J = 4.9 Hz, 2H) 3.87 (t, ^2J = 4.63 Hz, 2H), 1.43 (b, 1H). ^{19}F NMR ($CDCl_3$) δ, −75.6 (m, 2F), −87.3 (m, 2F). Anal. Calcd for $C_9H_5N_3O_3F_4$; C, 38.71; H, 1.81; N, 15.11. Found: C, 38.48; H, 1.85; N, 15.03.

The oil **8** (2.84 g, 9.9 mmol) was dissolved in acetone and then NaN_3 (0.650 g, 9.9 mmol) taken in water was added. The solution was refluxed overnight, cooled and mixed with water and extracted into ether, and dried over $MgSO_4$; the solvent was removed in a vacuum to produce yellow oil. The crude oil was purified by chromatography on a silica gel column using hexane and ethyl acetate in a 10: 3 ratio to obtain a yellow oil **10** (yield 75%). ^{1}H NMR ($CDCl_3$) δ, 1.42 (b, OH, 1H), 1.73 (m, 2H), 1.85 (m, 2H), 3.74 (t, ^2J = 6.2 Hz, 2H) 4.42 (t, ^2J = 6.4 Hz, 2H). ^{19}F NMR ($CDCl_3$) δ, −75.8 (m, 2F), −87.7 (m, 2F). Anal. Calcd for $C_{11}H_9N_3O_3F_4$; C, 42.99; H, 2.99; N, 13.71. Found: C, 42.64; H, 2.81; N, 13.64.

An equimolar solution of azido alcohol **9** (980 mg, 3.43 mmol) was slowly added to $PSCl_3$ (580 mg, 3.43 mmol) in $CHCl_3$ at 0° C with excess of Et_3N and refluxed for 8 h and monitored by ^{31}P NMR to the disappearance of $PSCl_3$ signals. The reaction mixture was cooled at 0° C and 2 molar equivalents of methyl hydrazine (360 μL, 6.86 mmol) were added slowly at 0 °C with continuous stirring. The solution was filtered, and the filtrate was evaporated and the mixture separated chromatographically on a silica gel column using hexane, ethyl acetate, and methanol in a 9:3:1 ratio to produce slightly yellow oil **11**, which solidifies on cooling (yield 75%). ^{1}H NMR ($CDCl_3$) δ, 2.84 (d, ^3J (P−H) = 11.1 Hz, 6H), 3.57 (b, 4H), 4.37 (t, ^2J = 4.2 Hz, 2H) 4.59 (t, ^2J = 4.3 Hz, 2H). ^{19}F NMR ($CDCl_3$) δ, −75.3 (m, 2F), −87.9 (m, 2F) ^{31}P NMR δ, 86.1. Anal. Calcd for $C_{11}H_{14}N_7F_4O_3PS$; C, 30.63; H, 3.27; N, 22.73. Found: C, 30.52; H, 3.19; N, 22.86.

Compound **12** was prepared in a similar manner using $POCl_3$.

Analytical data for **12** (yield 65%) ^{1}H NMR ($CDCl_3$) δ, 2.84 (d, ^3J (P−H) = 12.3 Hz, 6H) 3.81, (b, 4H) 4.22 (t, ^2J = 4.3 Hz, 2H), 4.62 (t, ^2J = 4.5 Hz, 2H), ^{19}F NMR ($CDCl_3$) δ, −75.8 (m, 2F), −88.5 (m, 2F). ^{31}P NMR δ, 18.5. Anal. Calcd for $C_{11}H_{14}N_7O_4F_4P$; C, 31.84; H, 3.41; N, 23.61. Found: C, 31.71; H, 3.36; N, 23.52.

A solution of the azido-modified alcohol **10** (1 g, 13.7 mmol) was refluxed with $PSCl_3$ (1.39 mL, 137 mmol) taken in $CHCl_3$ in the presence of excess of Et_3N and refluxed for 6 h, monitored by ^{31}P NMR spectroscopy. Two molar equivalents of methyl hydrazine was added to the above mixture cooled to 0 °C and stirred for an hour. The solution was filtered, and the filtrate was evaporated in a vacuum to yield pale yellow solid **13** (72% yield). ^{1}H NMR ($CDCl_3$) δ, 1.74 (m, 2H), 1.85 (m, 2H), 2.85 (d, ^3J (P−H) = 12.3 Hz, 6H), 3.41 (b, 4H), 3.72 (t, ^2J = 6.5 Hz, 2H), 4.38 (t, ^2J = 6.3 Hz, 2H). ^{31}P NMR δ, 86.2, ^{19}F NMR ($CDCl_3$) δ, −75.8 (m, 2F), −88.7 (m, 2F). Anal. Calcd for $C_{13}H_{18}N_7F_4O_3PS$; C, 33.99; H, 3.95; N, 21.34. Found: C, 33.88; H, 3.83; N, 21.25.

Compound **14** was prepared using a procedure similar to the one described with $POCl_3$. Analytical data for **14** (yield 82%) ^{1}H NMR ($CDCl_3$) δ, 1.78 (m, 2H), 1.81 (m, 2H), 2.81 (d, ^3J (P−H) = 12.4 Hz, 6H), 3.51 (b, NH_2, 4H), 3.75 (t, ^2J = 6.1 Hz, 2H), 4.31 (t, ^2J = 6.3 Hz) 2H). ^{31}P NMR δ, 18.8, ^{19}F NMR ($CDCl_3$) δ, −75.6 (m, 2F), −88.2 (m, 2F). Anal. Calcd for $C_{13}H_{18}N_7F_4O_4P$; C, 35.22; H, 4.09; N, 22.12. Found: C, 35.14; H, 4.18; N, 22.25.

General Synthesis of Pd Complexes of 6, 15−18. Equimolar concentrations of $PdCl_2(PhCN)_2$ and each of the amide-coupled perfluoroaryl phosphorus hydrazide **5** or perfluoroaryl azido functionalized methylene-bridged phosphorus hydrazides in CH_2Cl_2 (**11**−**14**) were mixed by adding $PdCl_2(PhCN)_2$ solution dropwise with slow stirring. The solution was evaporated partially and treated with hexane to precipitate the complex and filtered. The filtrate was washed with hexane (5 × 50 mL), dried, and dissolved in hot dry acetonitrile and cooled at −5 °C to yield orange red crystalline material. **Analytical data for 6:** (72% yield). ^{1}H NMR (CD_2Cl_2) δ, 3.15 (d, ^3J (P−H) = 12.1 Hz, 6H), ^{31}P NMR δ, 68.9 ^{19}F NMR (CD_2Cl_2) δ, −74.8 (m, 2F), −87.5 (m, 2F). Anal. Calcd for $C_9H_{11}N_8F_4OPS.PdCl_2$; C, 19.18; H, 1.97; N, 19.88. Found: C, 19.14; H, 1.90; N, 19.74. **Analytical data for 15:** (73% yield). ^{1}H NMR (CD_2Cl_2) δ, 3.12 (d, ^3J (P−H) = 11.3 Hz, 6H), 3.68 (b, 4H), 4.36 (t, ^2J = 4.2 Hz, 2H), 4.52 (t, ^2J = 4.5 Hz, 2H) ^{31}P NMR δ, 87.8 ^{19}F NMR (CD_2Cl_2) δ, −75.8 (m, 2F), −87.9 (m, 2F). Anal. Calcd for $C_{11}H_{14}N_7F_4O_3PSPdCl_2$; C, 21.71; H, 2.32; N, 16.11. Found: C, 22.01; H, 2.39; N, 16.21. **Analytical data for 16:** (82% yield). ^{1}H NMR (CD_2Cl_2) δ 2.76 (d, ^3J (P−H) = 10.1 Hz, 6H), 3.62 (b, 4H), 4.25 (t, ^2J = 4.4 Hz, 2H) 4.57 (t, ^2J = 4.3 Hz, 2H). ^{31}P NMR δ, 17.15 ^{19}F NMR (CD_2Cl_2) δ, −75.5 (m, 2F), −88.4 (m, 2F). Anal. Calcd for $C_{11}H_{14}N_7F_4O_4PPdCl_2$; C, 22.31; H, 2.38; N, 16.55. Found: C, 22.47; H, 2.45; N, 16.69. **Analytical data for 17:** (68% yield). ^{1}H NMR (CD_2Cl_2) δ, 1.72 (m, 2H), 1.84 (m, 2H), 2.67 (d, ^3J (P−H) = 12.8 Hz, 6H), 3.62 (b, 4H), 3.71 (t, ^2J = 6.3 Hz, 2H), 4.36 (t, ^2J = 6.2 Hz, 2H). ^{19}F NMR (CD_2Cl_2) δ, −76.1 (m, 2F), −87.4 (m, 2F). ^{31}P NMR, δ, 87.2 Anal. Calcd for $C_{13}H_{18}N_7F_4O_3PSPdCl_2$; C, 24.52; H, 2.85; N, 15.41. Found: C, 24.31; H, 2.95; N, 15.67. **Analytical data for 18:** (84% yield). ^{1}H NMR (CD_2Cl_2) δ, 1.68 (m, 2H), 1.84 (m, 2H), 2.65 (d, ^3J (P−H) = 12.8 Hz, 6H), 3.12 (b, 4H), 3.76 (t, ^2J = 6.4 Hz, 2H), 4.36 (t, ^2J = 6.1 Hz, 2H). ^{19}F NMR (CD_2Cl_2) δ, −75.4 (m, 2F), −88.7 (m, 2F). ^{31}P NMR δ, 17.2. Anal. Calcd for $C_{13}H_{18}N_7F_4O_4PPdCl_2$; C, 25.16; H, 2.92; N, 15.81. Found: C, 25.34; H, 2.88; N, 15.61.

Photochemical Synthesis of Cyclohexylamine Adducts 5a, 11a−16a. Separate solutions of perfluoroaryl azido phosphorus hydrazide derivatives (**5**, **11**−**16**) in a mixture of CH_2Cl_2 and cyclohexane (1:1) were exposed to a beam of light for 5 min in a quartz tube after bubbling with nitrogen for 2−3 min. The photochemical reaction was monitored by ^{19}F NMR spectroscopy to the destruction of the parent azide peaks. The solution was concentrated by evaporating solvent partially and dissolved in a minimum amount of methanol and separated on silica gel column using hexane, ethyl acetate, and methanol in a ratio of 9:2:1. In Pd complexes (**6a**, **15a**, and **16a**), the solvent was partially evaporated and reprecipitated using hexane. The precipitate was washed with hexane (3 × 10 mL) and dissolved in hot CH_3CN and evaporated slowly at room temperature to yield an orange yellow powder. **Analytical data for 5a:** (yield 73%). ^{1}H NMR ($CDCl_3$) δ, 4.7 (b, 1H), 2.96 (d, ^3J (P−H) = 10.2 Hz, 6H), 3.3 (b, 4H), 1.05−1.48 (m, 5H), 1.55−1.73 (m, 3H), 1.93−2.01 (m, 2H), 3.53 (m, 1H), 4.02 (s, 1H), ^{13}C (Dept), methine carbon δ, 51.8. ^{31}P NMR δ, 85.3, ^{19}F NMR ($CDCl_3$) δ, −77.2 (m, 2F), −99.8 (m, 2F). Anal. Calcd for $C_{15}H_{23}N_6F_4O_2PC$; 40.72; H, 5.24; N, 18.99. Found: C, 40.31; H, 5.57; N, 19.45. **Analytical data for 6a:** (yield 70% ± 5). ^{1}H NMR (CD_2Cl_2) δ, 4.6 (b, 1H), 3.12 (d, ^3J (P−H) = 11.5 Hz, 6H), 3.6 (b, s, NH_2, 4H), 1.06−1.41 (m, 5H), 1.59−1.76 (m, 3H), 1.95−2.02 (m, 2H), 3.56 (m, 1H), 4.06 (s, 1H), ^{13}C (Dept), methine carbon δ, 53.4. ^{31}P NMR δ, 87.8 ^{19}F NMR ($CDCl_3$) δ, −77.25(m, 2F), −99.2 (m, 2F). Anal. Calcd for $C_{15}H_{23}N_6F_4OPSPdCl_2$; C, 29.07; H, 3.74; N, 13.56. Found: C, 29.28; H, 3.57; N, 13.21. **Analytical data for 11a:** (yield 90%). ^{1}H NMR ($CDCl_3$), δ 2.84 (d, ^3J (P−H) = 11.1 Hz, 6H), 3.52 (b, 4H), 4.37 (t, ^2J = 4.2 Hz, 2H) 4.59 (t, ^2J = 4.3 Hz, 2H), 1.05−1.45 (m, 5H), 1.56−1.77 (m, 3H), 1.91−2.04 (m, 2H), 3.56 (m, 1H), 4.05 (s, 1H), ^{13}C (Dept), methine carbon δ, 52.4, ^{19}F NMR ($CDCl_3$) δ, −76.5 (m, 2F), −99.8 (m, 2F) ^{31}P NMR δ, 86.5. Anal. Calcd for $C_{17}H_{26}N_5O_3F_4PS$; C, 41.89; H, 5.38; N, 14.37. Found: C, 41.85; H, 5.28; N, 14.33. **Analytical data for 12a:** (yield 93%). ^{1}H NMR ($CDCl_3$) δ, 2.83 (d, ^3J (P−H) = 12.3 Hz) 6H) 3.80, (b, 4H) 4.22 (t, ^2J = 4.3 Hz, 2H), 4.61 (t, ^2J = 4.5 Hz, 2H), 1.05−1.45 (m, 5H), 1.54−1.77 (m, 3H), 1.92−2.04 (m, 2H), 3.56 (m, 1H), 4.03 (s, 1H), ^{13}C (Dept), methine carbon δ, 53.1. ^{19}F NMR ($CDCl_3$) −77.7 (m, 2F), −99.8 (m, 2F). ^{31}P NMR δ, 18.9. Anal. Calcd for $C_{17}H_{26}N_5O_4F_4P$; C, 43.31; H, 5.56; N, 14.86. Found: C, 43.35; H, 5.51; N, 14.88. **Analytical data for 13a:** (yield 92%). ^{1}H NMR ($CDCl_3$) δ, 1.73 (m, 2H), 1.83 (m, 2H), 2.83 (d, ^3J (P−H) = 12.3 Hz, 6H), 3.40 (b, 4H), 3.72 (t, ^2J = 6.5 Hz, 2H), 4.36 (t, ^2J = 6.3 Hz, 2H) 1.09−1.42 (m, 5H), 1.61−1.77 (m, 3H), 1.96−2.05 (m, 2H), 3.55 (m, 1H), 4.04 (s, 1H), ^{13}C (Dept), methine carbon δ, 52.7. ^{31}P NMR δ, 86.8, ^{19}F NMR ($CDCl_3$) δ, −77.1(m, 2F), −99.6 (m, 2F). Anal. Calcd for $C_{19}H_{30}N_5F_4O_3PS$; C, 44.27; H, 5.87; N, 13.59. Found: C, 44.31; H, 5.83; N, 13.61. **Analytical data for 14a:** (yield 89%). ^{1}H NMR ($CDCl_3$) δ, 1.76 (m, 2H), 1.82 (m, 2H), 2.81 (d, ^3J (P−H) = 12.4 Hz, 6H), 3.56 (b, 4H), 3.75 (t, ^2J = 6.1 Hz, 2H), 4.31 (t, ^2J = 6.3 Hz) 2H), 1.08−1.45 (m, 5H), 1.56−1.75 (m, 3H), 1.95−2.04 (m, 2H), 3.59 (m, 1H), 4.05 (s, 1H), ^{13}C (Dept), methine carbon δ, 51.9. ^{31}P NMR δ, 18.2, ^{19}F NMR

($CDCl_3$) δ, −77.4 (m, 2F), −99.3 (m, 2F). Anal. Calcd for $C_{19}H_{30}N_5F_4O_4P$; C, 45.69; H, 6.05; N, 14.02. Found: C, 45.63; H, 6.08; N, 14.08. **Analytical data for 15a:** (yield 74 ± 5%). ^{1}H NMR (CD_2Cl_2) δ, 3.12 (d, ^3J (P−H) = 12.1 Hz, 6H), 3.67 (b, 4H), 4.25 (t, ^2J = 4.2 Hz, 2H) 4.57 (t, ^2J − 4.3 Hz, 2H), 1.03−1.45 (m, 5H), 1.61−1.79 (m, 3H), 1.94−2.05 (m, 2H), 3.56 (m, 1H), 4.07 (s, 1H), ^{13}C (Dept), methine carbon δ, 53.1. ^{31}P NMR δ, 87.3 ^{19}F NMR (CD_2Cl_2) δ, −77.1 (m, 2F), −99.2 (m, 2F). Anal. Calcd for $C_{17}H_{26}N_5F_4O_3PSPdCl_2$; C, 30.72; H, 3.94; N, 10.53. Found: C, 30.85; H, 3.99; N, 10.62. **Analytical data for 16a:** (yield 75 ± 5%). ^{1}H NMR (CD_2Cl_2) δ, 2.75 (d, ^3J (P−H) = 10.1 Hz, 6H), 3.60 (b, 4H), 4.25 (t, ^2J = 4.2 Hz, 2H) 4.56 (t, ^2J = 4.3 Hz, 2H), 1.07−1.45 (m, 5H), 1.57−1.78 (m, 3H), 1.91−2.06 (m, 2H), 3.55 (m, 1H), 4.06 (s, 1H), ^{13}C (Dept), methine carbon δ, 53.1. ^{31}P NMR δ, 17.3 ^{19}F NMR (CD_2Cl_2) δ, −77.9 (m, 2F), −99.7 (m, 2F). Anal. Calcd for $C_{17}H_{26}N_5F_4O_4PPdCl_2$; C, 31.48; H, 4.04; N, 10.79. Found: C, 31.42; H, 4.08; N, 10.81.

Photochemical Reaction of Hydrazones 1, 2, and 4 in Cyclohexane. All the hydrazones **1, 2**, and **4** were dissolved in either neat cyclohexane or in a mixture of cyclohexane and CH_2Cl_2 (1:1) and photolyzed in NMR tube for 30−60 min. The photochemical reactions were monitored by ^{19}F NMR spectroscopy. All the Schiff base compounds yielded triplet-derived amines in high yields after photolysis. **Analytical data for 1b:** (yield 87%). ^{1}H NMR ($CDCl_3$) δ, 2.93 (d, 3H), 3.3 (b, 1H), 4.2 (b, 2H), 7.5 (s, 1H), ^{19}F NMR ($CDCl_3$) δ, −84.6 (m, 2F), −101.8 (m, 2F). **Analytical data for 2b:** (yield 82%). ^{1}H NMR ($CDCl_3$) δ, 2.95 (d, ^3J = 11.8 Hz, 3H), 3.25 (d, ^3J = 8.4 Hz, 3H), 3.3 (b, 2H), 4.3 (b, 2H), 7.1−7.8 (m, 5H) 7.7 (s, 1H), ^{19}F NMR ($CDCl_3$) δ, −82.3 (m, 2F), −101.6 (m, 2F), ^{31}P NMR ($CDCl_3$) δ, 82.5. **Analytical data for 4b:** (yield 82%). ^{1}H NMR ($CDCl_3$) δ, 2.94 (d, ^3J = 11.6 Hz., 3H), 4.2 (b, 2H), 7.0−7.5 (m, 10 H) 7.6 (s, 1H), ^{19}F NMR ($CDCl_3$) δ, −82.1 (m, 2F), −101.7 (m, 2F), ^{31}P NMR δ, −5.3. The analytical data for the compound above coincides well with the triplet-derived product from the photolysis of **4** in methanol.

Photochemical Reaction of 1, 2, and 4 with Pyridine. All compounds were dissolved in a mixture of CH_2Cl_2 and pyridine (8:2) and degassed by bubbling with nitrogen for 5 min. The solution was photolyzed for 2−5 min. The solution turned from light yellow to a persistent amber color shifting the absorption maximum to the visible range 390−415 nm for all the compounds. This is a typical absorption of a pyridine ylide.[15]

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Supporting Information Available: Radiochemical characterization and purity of ^{99m}Tc complex, additional flash photolysis data are available as supplementary information (8 pages). This material is contained in libraries on microfische, immediately follows the article in the microfilm version of the journal, and can be ordered from the ACS. See any current masthead page for ordering information.

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