JOC*Article*

Chemistry of Bifunctional Photoprobes. 6. Synthesis and Characterization of High Specific Activity Metalated Photochemical Probes: Development of Novel Rhenium Photoconjugates of Human Serum Albumin and Fab Fragments

Raghavan Rajagopalan,[†] Robert R. Kuntz,[‡] Uday Sharma,[‡] Wynn A. Volkert,[§] and
Raghoottama S. Pandurangi*,[‡]

Department of Chemistry, University of Missouri, Columbia, Missouri 65211, Gliatech, 23420,
Commerce Park Road, Beachwood, Ohio 44122, and Research Service, VA Hospital,
University of Missouri, Columbia, Missouri 65211

r_pandurangi@diatide.com

Received August 1, 2001

Functionalization of perfluoro aryl azides by bifunctional chelating agents (BFCAs) capable of forming high specific activity complexes with ^{99m}Tc (for γ-imaging) and ^{188}Re (for radiotherapy) is described. The synthesis of multidonor BFCAs containing N_2S_2, N_4, and N_3S donor groups containing imidazole, pyridine, and pyrazine functionalities that may be important for tuning the pharmacokinetic parameters is also described. Functionalization of perfluoro aryl azides at various sites on BFCAs yields novel bifunctional photolabile chelating agents (BFPCAs) that are useful for covalent attachment to biomolecules. A representative Re–BFPCA **8a** in a model solvent, diethylamine, proceeded to give a high yield of intermolecular NH insertion product without the decomplexation of the metal ion from **8a**. All products originated from the photolysis of **8a** in diethylamine are characterized by analytical techniques, and a plausible mechanism of formation of different photolytic products is suggested. The high yield of intermolecular NH insertion of Re–BFPCA **8a** is extended to labeling of human serum albumin (HSA) and Fab fragments under aqueous conditions. The photolabeling technology developed here offers a new way to attach diagnostically and therapeutically useful radiotracers (e.g., ^{99m}Tc, ^{188}Re) to Fab fragments for potential noninvasive imaging and therapy of cancer.

Introduction

Conjugation of radiolabeled molecular probes to antibodies and antibody fragments (Fab, $F_{(ab')2}$, and F_{bs}) is a versatile technique to target the molecular probes to specific biological sites either for imaging or for therapy of cancer.[2,3] Radioimmunoconjugates based on ^{99m}Tc and its congeners, ^{186}Re and ^{188}Re, continue to attract much attention in the diagnosis of cancer[4,5] and cardiovascular diseases[6] due to their favorable nuclear properties (^{99m}Tc, γ, 140 keV, $t_{1/2} = 6$ h; ^{186}Re, β, 1.07 MeV, $t_{1/2} = 90$ h, ^{188}Re, β, 2.21 MeV, $t_{1/2} = 17$ h).[7,8] Fab fragments are, in general, better carriers of radiotracers to tumors than the whole antibody because of the observed improvement in the image quality of tumors resulting from relatively fast clearance of the radiolabeled fragments from nontarget sites.[9–11]

Most conjugation methods developed thus far for peptides and proteins have focused on attaching the bifunctional chelates (BFCAs) at the N-terminal amino group, ϵ-amino group of lysine, C-terminal carboxyl group, and carboxyl groups of aspartic and glutamic acids.[12] However, these functional groups may be essential for bioactivity, and any alterations of these

---

* To whom correspondence should be addressed. Fax: 603-437-8977.

[†] Gliatech.

[‡] Department of Chemistry, University of Missouri.

[§] VA Hospital, University of Missouri.

(1) Part 5: Pandurangi, R. S.; Rao, S. N.; Piotr, C.; Stillwell, L. I.; Barnes, C. L.; Kuntz, R. R. Chemistry of bifunctional photoprobes. Part 5: Synthesis of high specific activity ACE inhibitor metal complex for diagnosis of tissue repair: Conformation analysis of lisinopril derivatives by molecular dynamics studies. *J. Peptide Res.* **2002**, submitted for publication.

(2) (a) Schubiger, P. A.; Alberto, R.; Smith, A. *Bioconjugate Chem.* **1996**, *7*, 165–179. (b) Wilber, D. S. *Bioconjugate Chem.* **1992**, *3*, 433–469. (c) Koppel, G. A., *Bioconjugate Chem.* **1990**, *1*, 13–23. (d) Fritzberg, A. R.; Wilber, D. S. In *Targeted Delivery of Imaging Agents*; Torchilin, V. P., Ed.; CRC Press: Boca Raton, FL, 1995; pp 83–101. (e) Griffiths, G. L.; Goldenberg, D. M.; Jones, A. L.; Hanson, H. J. *Bioconjugate Chem.* **1992**, *3*, 91–99. (f) Johannsen B.; Spies, H. Technetium(V) chemistry as relevant to nuclear medicine. *Top. Curr. Chem.* **1996**, *176*, 79–121. (g) Hjelstuen, O. K. Technetium-99m chelates in nuclear medicine. *Analyst* **1995**, *120*, 863–866.

(3) (a) Kowalsky, R. J.; Perry, J. R. In *Chemistry of Radiopharmaceuticals. Radiopharmaceuticals in nuclear Medicine Practice*; Baum, S., Ed.; Appleton & Lange: Norwalk, CT, pp 75–95. (b) Goldenberg, D. M.; Deland, F. H.; Kim, E. E.; Bennette, S.; Primus, F. J.; Van Nagel, J. R.; Estes, N.; Desimone, P.; Rayburn, P. *N. Engl. J. Med.* **1978**, *298*, 1384. (c) Goldenberg, D. M.; Kim, E. E.; Deland, F. H.; Bennette, S.; Primus, F. J. *Cancer Res.* **1980**, *40*, 2984. (d) Cesati, R. R., III; Katzenellenbogen, J. A. *J. Am. Chem. Soc.* **2001**, *123*, 4093–4094. (e) Skaddan, M. B.; Wust, F. R.; Jonson, S.; Syhre, R.; Welch, M. J.; Spies, H.; Katzenellenbogen, J. A. *Nucl. Med. Biol.* **2000**, *27*, 269–278. (f) Skaddan, M. B.; Wuest, F. R.; Katzenellenbogen, J. A. *J. Org. Chem.* **1999**, *64*, 8108–8121. (g) Wust, F.; Skaddan, M. B.; Leibnitz, P.; Spies, H.; Katzenellenbogen, J. A.; Johannsen, B. *Bioorg. Med. Chem.* **1999**, *7*, 1827–1835. (h) Wust, F.; Carlson, K. E.; Katzenellenbogen, J. A.; Spies, H.; Johannsen, B. *Steroids* **1998**, *63*, 665–671.

10.1021/jo010782u CCC: $22.00

residues may potentially result in loss of affinity of the antibody or of its fragments to the target tissue. For example, labeling antibodies at the ϵ-amino groups, which are located near the antigen-binding sites, has often resulted in low bioconjugation yield and loss of antibody specificity of the eventual bioconjugates.[13] In contrast, direct attachment of BFCAs at the unactivated positions in the hydrocarbon side chain such as valine, leucine, phenylalanine, etc. has not been extensively investigated. This approach would be very desirable because of two principal reasons: (a) it would preserve the functional groups needed for binding antigens, and (b) it would obviate the need for protecting group manipulations in the carrier molecules.

However, covalent attachment of molecular probes to hydrocarbon side chains is challenging and requires activation of CH bonds under buffer conditions. The photolabeling method does not require specific functional groups to be present in the biomolecule since labeling can be achieved by activating the C$-$H bond of hydrophobic residues. Singlet nitrenes are versatile CH activating agents in model solvents, studied by Platz et al.[14] and Keena et al.,[15] and we have extended the studies to incorporate ^{99m}Tc metal for labeling biomolecules in our laboratory.[16] Successful utility of the photolabeling technique in nuclear medicine depends on several factors including (a) selection of high specific activity BFCA, (b) the ease of preparation of ^{99m}Tc and Re complexes, and (c) preservation of the photochemical moiety (N_3) and its intermolecular insertion properties after metal complexation. Recently, we have reported the synthesis and photoattachment of BFPCAs with ^{99m}Tc to human serum albumin (HSA)[17] and to immunogloblin[18] on the basis of the photochemistry of perfluoro aryl azides. While the reported BFPCAs were shown to insert into the hydrophobic part of these biomolecules with high efficiency and with retention of bioactivity, they could not be prepared in high specific activity required for eventual clinical use.

Previous studies on the preparation of technetium$-$mercaptoacetyl glycylglycylglycine (^{99m}Tc$-$MAG$_3$) complexes at very high specific activity (i.e., 3 Ci/mmol)[19-24] and on the favorable pharmacokinetic properties of some

---

(4) (a) Volkert, W. A.; Hoffman, T. J. *Chem. Rev.* **1999**, *99*, 2269−2292. (b) Spencer, R. P.; Suvers, R. H.; Friedman, A. M. *Radionuclides in Therapy*; CRC Press: Boca Raton, FL, 1987. (c) Kaplan, E. In *Therapy in Nuclear Medicine*; Spencer, R., Ed.; Grune & Straton: New York, 1978; p 237. (d) Herbert, J. C. *Nuclear Medicine Therapy*; Thieme Medical Publishers: New York, 1987. (e) Fritzberg, A. R.; Gutavson, L. M.; Hylarides, M. D.; Reno, J. M. *Chemical and Structural Approaches to Rational Drug Design*; Weiner, D. B., Williums, M. V., Eds.; CRC Press: Boca Raton, FL, 1995; Chapter 5, p 125.

(5) (a) Edwards, D. S. *Chem. Rev.* **1999**, *99*, 2235−2268. (b) Hom, R. K.; Katzenellenbogen, J. A. *Nucl. Med. Biol.* **1997**, *24*, 485−498. (c) Hunter, D. H.; Luyt, L. G. *Bioconjugate Chem.* **2000**, *11*, 175−181. (d) Luyt, L. G.; Jenkins, H. A.; Hunter, D. H. *Bioconjugate Chem.* **1999**, *10*, 470−479. (e) Polyakov, V.; Sharma, V.; Dahlheimer, J. L.; Pica, C. M.; Luker, G. D.; Piwnica-Worms, D. *Bioconjugate Chem.* **2000**, *11*, 762−771. (f) Yamamura, N.; Magata, Y.; Arano, Y.; Kawaguchi, T.; Ogawa, K.; Konishi, J.; Saji, H. *Bioconjugate Chem.* **1999**, 10, 489−495. (g) Baldas, J. The coordination chemistry of Technetium. In *Advances in Inorganic Chemistry*; 1999; Vol. 41, pp 1−123. (h) Barrera, J.; Burrell, A. K.; Bryan J. C. *Inorg. Chem.* **1996**, 35, 335−341. (i) Alberto, R. High and low valancy organometallic compounds of Technetium and Rhenium. *Top. Curr. Chem.* **1996**, *176*, 149−187. (j) Volkert, W. A.; Jurisson, S. Technetium-^{99m} chelates as radiopharmaceuticals. Technetium and Rhenium their chemistry and its applications. In *Topics in Current Chemistry*; Yoshihara, K., Omori, T., Eds.; Springer Verlag: Berlin, Heidelberg, 1996; Chapter 4, pp 123−148. (k) Jurisson, S.; Berning, D.; Jia, W.; Ma, D. *Chem. Rev.* **1993**, *93*, 1137.

(6) (a) Bellar, G. A.; Zaret, B. L. *Circulation* **2000**, *101*, 1465. (b) Bellar, G. A. *Adv. Intern. Med.* **1997**, *201*, 139. (c) Bellar, G. A. Current status of nuclear cardiology techniques. *Curr. Probl. Cardiol.* **1991**, *535*, 451. (d) Maddahi, J.; Kiat, H.; Friedman, J. D.; Berman, D. S.; Van Train, K. F.; Garcia, E. V. Technetoum-99m-sestamibi myocardial perfusion imaging for evaluation of coronary artery disease. In *Nuclear Cardiology: State of the art and future applications*, 1st ed.; Zaret, B. L., Beller, G. A., Eds.; Mosby-yearbook: St. Louis, MO, 1993; Vol. 200, p 191.

(7) (a) Ehrhardt, G. J.; Ketring, A. R.; Volkert, W. A. A production of isotopes at nuclear reactors. In *Synthesis and Applications of Isotopically Labeled Compounds*; Buncel E., Kabalka, G. W., Eds.; Elsevier Science: Dordrecht, The Netherlands, 1991; pp 159−164. (b) Goswami, N.; Alberto, R.; Barnes, C. L.; Jurisson, S. *Inorg. Chem.* **1996**, *35*, 7546.

(8) (a) Griffiths, G. L.; Goldenberg, D. M.; Roesch, F.; Hansen, H. J. *Clin. Cancer Res.* **1999**, *5*, 3001s−3003s. (b) Arano, Y.; Fujioka, Y.; Akizawa, H.; Ono, M.; Masahiro, Uehara, T.; Wakisaka, K.; Nakayama, M.; Sakahara, H.; Konishi, J.; Saji, H. *Cancer Res.* **1999**, *59*, 128−134. (c) Spradau, T. W.; Katzenellenbogen, J. A. *Bioorg. Med. Chem.* Lett. **1998**, *8*, 3235−3240. (d) Skaddan, M. B.; Katzenellenbogen, J. A. *Bioconjugate Chem.* **1999**, *10*, 119−129. (e) Minutolo, F.; Katzenellenbogen, J. A. *J. Am. Chem. Soc.* **1998**, *120*, 13264−13265. (f) Hom, R. K.; Katzenellenbogen, J. A. *J. Org. Chem.* **1997**, *62*, 6290−6297. (g) Katzenellenbogen, J. A.; Welch, M. J.; Dehdashti, F. *Anticancer Res.* **1997**, *17*, 1573−1576.

(9) (a) Schmidt, P. F.; Smith, S. V.; Bundese, P. G. *Nucl. Med. Biol.* **1998**, *25*, 639−649. (b) Goldenberg, D. M.; Juweid, M.; Dunn, R. M.; Sharkey, R. M. *J. Nucl. Med. Technol.* **1997**, *25*, 18−23. (c) Ultee, M. E.; Bridger, G. J.; Abrams, M. J.; Longley, C. B.; Burton, C. A.; Larsen, S. K.; Hanson, G. W.; Padmanabhan, S.; Gaul, F. E.; Schwartz, D. A. *J. Nucl. Med.* **1997**, *38*, 133−138. (d) Bridger, G. J.; Abrams, M. J.; Padmanabhan, S.; Gaul, F.; Larsen, S.; Henson, G. W.; Schwartz, D. A.; Longley, C. B.; Burton, C. A.; Ultee, M. E. *Bioconjugate Chem.* **1996**, *7*, 255−264. (e) Rajagopalan, R, Srinivasan, A. Mallinckrodt Medical Inc. US Patent 5633372, 1993. (f) Tsai, S. W.; Li, L.; Williums, L.E.; Anderson, A. L.; Raubitschek, A. A.; Shively, J. E. *Bioconjugate Chem.* **2001**, *12*, 264.

(10) Karacay, H.; McBride, W. J.; Griffiths, G. L.; Sharkey, R. M.; Barbet, J.; Hansen, H. J.; Goldenberg, D. M. *Bioconjugate Chem.* **2001**, *12*, 264.

(11) Amato, R.; Kim, E.; Edmund, P.; Debra, A.; Dimitros, K.; Leela, P. J. *Cancer Res. Clin. Oncol.* **2000**, *126*, 161.

(12) (a) Law, K. L.; Studmann, K. E.; Carlson, R. E.; Swanson, T. A.; Buirge, A. W.; Ahmad, A. *Anticancer Res.* **1990**, *10*, 845. (b) Gene, M. D.; Walker M. A. *Pharmacol. Therapeut.* **1999**, *83*, 67.

(13) (a) Diwanji, M. K. The chemistry of 99mTc labeled radiopharmaceuticals. In *Seminars in Nuclear Medicine*, V XX; Freeman, L. M., Blaufox, M. D., Eds.; W. B. Saunders: Philadelphia, pp 5−27. (b) Li, M.; Mears, C. F.; Zhong, G. R.; Miers, L.; Xiong, C. Y.; DeNardo, S. J. *Bioconjugate Chem.* **1994**, *5*, 101.

(14) (a) Poe, R., J. Grayzar; Young, M. J. T.; Leyva, E.; Schnap, K. A.; Platz, M. S. *Bioconjugate Chem.* **1993**, *4*, 172. (b) Poe, R.; Schnap, K. A.; Young, M. J. T.; Grayzar, J.; Platz, M. S. *J. Am. Chem. Soc.* **1992**, *114*, 5054.

(15) (a) Keana, J. F. W.; Cai, S. X. *J. Org. Chem.* **1990**, *55*, 3640. (b) Cai, S. X.; Glenn, D. J.; Keana, J. F. W. *J. Org. Chem.* **1992**, *57*, 1299.

(16) (a) Pandurangi, R. S.; Katti, K. V.; Stilwell, L.; Barnes, C. L. *J. Am. Chem. Soc.* **1998**, *120*, 11364. (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.; Kuntz, R. R.; Volkert, W. A. *App. Rad. Isotopes* **1995**, *46*, 233.(e) Pandurangi, R. S.; Karra, S. R.; Katti, K. V.; Volkert, W. A.; Kuntz, R. R. *J. Org. Chem.* **1997**, *62*, 2587.(f) Pandurangi, R. S.; Katti, K. V.; Volkert, W. A.; Kuntz, R. R. *Inorg. Chem.* **1996**, *35*, 3716. (g) Pandurangi, R. S.; Lusiak, P.; Yao S.; Weber, K. T.; Kuntz, R. R. *Bioorg. Chem.* **1997**, *25*, 77−87. (h) Pandurangi, R. S.; Karra, S. R.; Kuntz, R. R.; Volkert, W. A. *Photochem. Photobiol.* **1997**, *65*, 101.

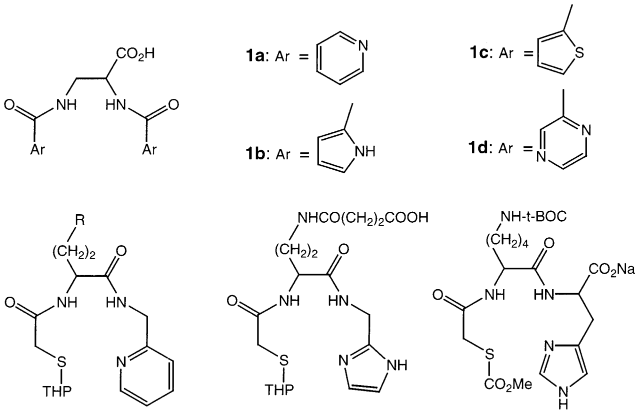
(17) Pandurangi, R. S.; Lusiak, P.; Platz, M. S.; Volkert, W. A. Kuntz R. R. *J. Org. Chem.* **1998**, *63*, 9019.

(18) Pandurangi, R. S.; Karra, S. R.; Kuntz, R. R.; Volkert, W. A. *Photochem. Photobiol.* **1996**, *64*, 100.

(19) (a) Rajagopalan, R.; Grummon, G. D.; Bugaj, J.; Halleman, L. S.; Webb, E. G.; Marmion, M. E.; Vanderheyden, J. L.; Srinivasan, A. *Bioconjugate Chem.* **1997**, *8*, 407−415. (b) Hom, R. K.; Katzenellenbogen, J. A. *J. Org. Chem.* **1997**, *62*, 6290−6297. (c) Grummon, G.; Rajagopalan, R.; Palenik, G. J.; Koziol, A. E.; Nosco, D. L. *Inorg. Chem.* **1995**, *34*, 1764−1772. (d) Rao, T. N.; Adhikesavaln, D.; Camerman, A.; Fritzberg, A. R. *J. Am. Chem. Soc.* **1990**, *112*, 5798−5804. (e) Rao, T. N.; Adhikesavalu, D.; Camerman, A.; Fritzberg, A. R. *Inorg. Chim. Acta* **1991**, *180*, 63−67. (f) Bell, R. A.; McCarry, B. E.; Valliant, J. F. *Inorg. Chem.* **1998**, *37*, 3517−3520.

(20) Pietzsch, H. J.; Gupta, A.; Reisgys, M.; Drews, A.; Seifert, S.; Syhre, R.; Spies, H.; Alberto, R.; Abram, U.; Schubiger, P. A.; Johannesen, B. *Bioconjugate Chem.* **2000**, *11*, 414−424.

CHART 1

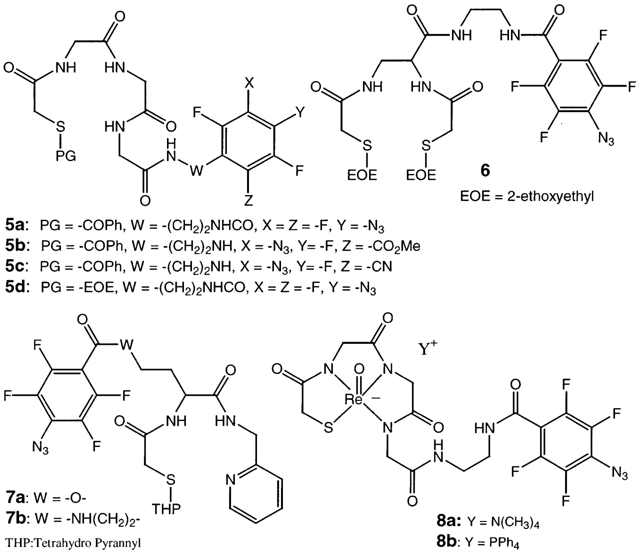


2a: R = -OCO(CH$_2$)$_2$CO$_2$H
2b: R = -(CH$_2$)$_2$NH$_2$

3

4

CHART 2

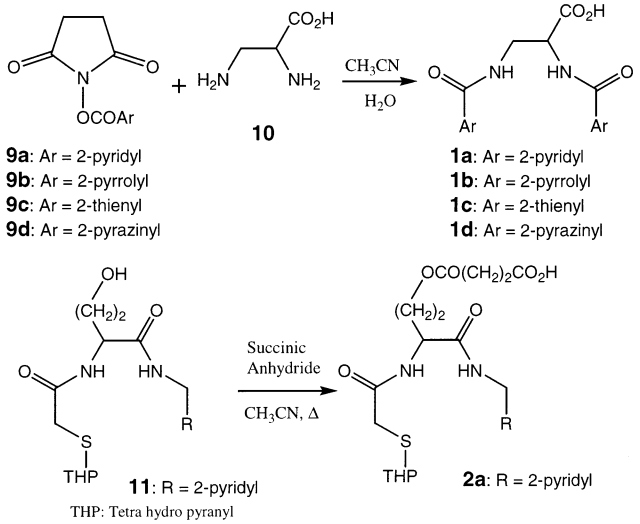


5a: PG = -COPh, W = -(CH$_2$)$_2$NHCO, X = Z = -F, Y = -N$_3$
5b: PG = -COPh, W = -(CH$_2$)$_2$NH, X = -N$_3$, Y= -F, Z = -CO$_2$Me
5c: PG = -COPh, W = -(CH$_2$)$_2$NH, X = -N$_3$, Y= -F, Z = -CN
5d: PG = -EOE, W = -(CH$_2$)$_2$NHCO, X = Z = -F, Y = -N$_3$

7a: W = -O-
7b: W = -NH(CH$_2$)$_2$-
THP:Tetrahydro Pyrannyl

8a: Y = N(CH$_3$)$_4$
8b: Y = PPh$_4$

SCHEME 1



9a: Ar = 2-pyridyl
9b: Ar = 2-pyrrolyl
9c: Ar = 2-thienyl
9d: Ar = 2-pyrazinyl

1a: Ar = 2-pyridyl
1b: Ar = 2-pyrrolyl
1c: Ar = 2-thienyl
1d: Ar = 2-pyrazinyl

11: R = 2-pyridyl

2a: R = 2-pyridyl

THP: Tetra hydro pyranyl

heterocyclic N$_3$S metal complexes[19a] prompted us to explore novel MAG$_3$-type photochemical probes as well as other nitrogen−sulfur ligands with different chelating motifs (e.g., N$_3$S, N$_2$S$_2$, N$_4$, etc.), which can be used to tune the lipophilicity of the probe and to provide different coordination modes for Tc and Re to achieve high stability, high specific activity, and optimium pharmacokinetics[25−27] in vivo. In general, ^{99m}Tc−MAG$_3$ is formed through the deprotection of sulfur at high temperature.[28−33] The ease of formation of ^{99m}Tc complexes depends on the ease of removal of the protecting group on sulfur.

High temperature condition is clearly undesirable due to thermal sensitivity of the biomolecule involved in labeling.

Thus, as a part of our research efforts directed at the development of efficient photochemical bioconjugate probes, herein we describe the synthesis of some novel heterocyclic ligands **1**−**4** (Chart 1) and of the photoprobes **5**−**8** (Chart 2); present the results pertaining to rhenium metal complexation, photochemistry, and photoconjugation of rhenium-chelated photoprobe **8**; and provide some insight on the possible mechanism of N−H insertion into the biomolecules by establishing the photochemistry of the Re complex at the macroscopic level using diethylamine as a model solvent. Photochemistry of the Re−MAG$_3$ photoprobe (**8a**) in diethylamine and conjugation of Re−photoprobe to HSA and Fab fragments constitutes the first report of the metal remaining intact during the photoconjugation process.

Results and Discussion

I. Synthesis of Bifunctional Chelating Agents (BFCAs) and Photoprobes. The synthesis of heterocyclic N$_4$ ligands is shown in Scheme 1. Ligands **1a**−**d** were prepared by acylation of 2,3-diaminopropionic acid (**10**) with the active esters **9a**−**d** derived from pyridine, pyrrole, thiophene, and pyrazine, respectively. Synthesis of N$_3$S ligands (**2**−**4**) are shown in Schemes 1 and 2. The pyridyl ligand **2a** was prepared by the reaction of the

---

(21) (a) VanGog, F. B.; Visser, G. W. M.; Klok, R.; VanDer Schors, R.; Snow, G. B.; Van Dongen, G. A. M. S. *J. Nucl. Med.* **1996**, *37*, 352−362. (b) Hanson, L.; Taylor, A. Jr.; Marzilli, L. G. *Metal-Based Drugs* **1994**, *1*, 31−39. (c) Canney, D. J.; Billings, J.; Franscesconi, L. C.; Guo, Y. Z.; Haggerty, B. S.; Rheingold, A. L.; Kung, H. F. *J. Med. Chem.* **1993**, *36*, 1032−1040.

(22) (a) Noll, B.; Johhansen, B.; May, K.; Spies, H. *Appl. Radiat. Isot.* **1992**, *43*, 899−901. (b) Hansen, L.; Cini, R.; Taylor, A., Jr.; Marzilli, L. G. *Inorg. Chem.* **1992**, *31*, 280.

(23) O'Neil, J. P.; Carlson, K. E.; Anderson, C. J.; Welch, M. J.; Katzenellenbogen, J. A. *Bioconjugate Chem.* **1994**, *5*, 182−193.

(24) Fritzberg, A. R.; Abrams, P. G.; Beanmier, P. L.; Kasina, S.; Charles Morgan, A.; Rao, T. N.; Reno, J. M.; Sanderson, J. A.; Srinivasan, A.; Scott Wilbur, D. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4025−4029.

(25) John Lister J.; Moyer, B. R.; Dean, R. T. Q. *J. Nucl. Med.* **1997**, *41*, 111.

(26) Scott Edwards, D.; Liu, S.; Barett, J. A.; Harris, A. R.; Looby, R. J.; Aiegler, M. C.; Heminway, S. J.; Carrol, T. R. *Bioconjugate Chem.* **1997**, *8*, 146.

(27) Dilworth, J. R.; Parrott, S. J. *Chem. Soc. Rev.* **1998**, *27*, 43.

(28) (a) Srinivasan, A. Method of radiolabeling chelating compounds comprising sulfur atoms with metal radionuclides. US Patent No. 5,021,556, 1991. (b) Kasina, S.; Rao, T. N.; Srinivasan, A.; Sanderson, J. A.; Fitzner, J. N.; Reno, J. M.; Beaumier, P. L.; Fritzberg, A. R. *J. Nucl. Med.* **1991**, *32*, 1445.

(29) Sugiyura, Y. *Inorg. Chem.* **1978**, *17*, 2176.

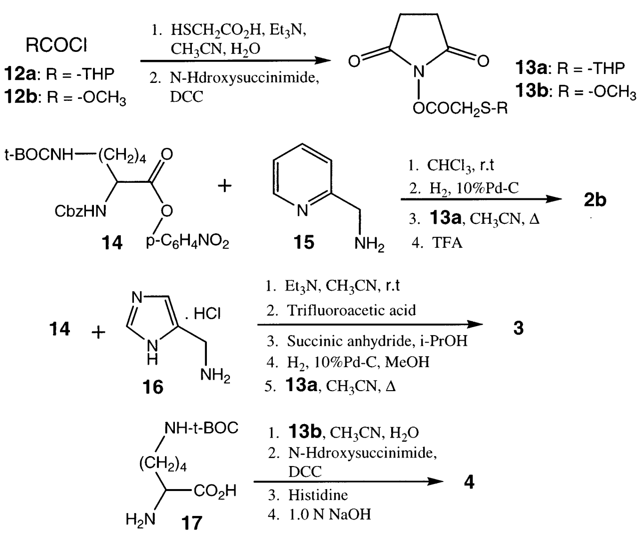
(30) Bryson, N.; Lister-James, J.; Jones, A. G.; Davis, W. M.; Davidson, A. *Inorg. Chem.* **1990**, *29*, 2948.

(31) Mather, S. H.; Elison, D. *J. Nucl. Biol. Med.* **1994**, *38*, 481.

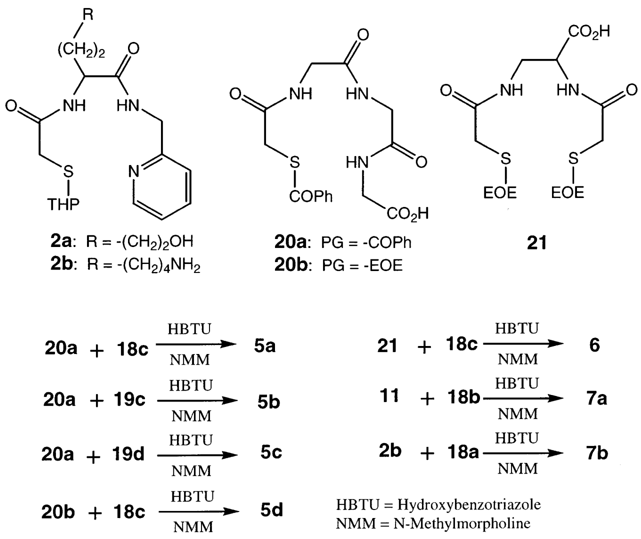
(32) (a) Das. T., Banerjee, S.; Samuel, G.; Kothari, K.; Unni, P. R.; Sarma, H. D.; Ramamoorthi, N.; Pillai, M. R. A. *Nucl. Med. Biol.* **2000**, *27*, 189−197.

(33) Kniess, T.; Noll, S.; Noll, B.; Spies, H.; Johansen, B. *J. Radioanal. Nucl. Chem.* **1999**, *240*, 657−660.

SCHEME 2

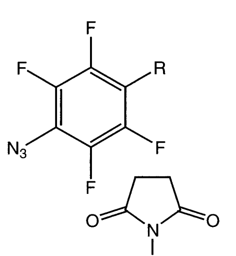


SCHEME 3

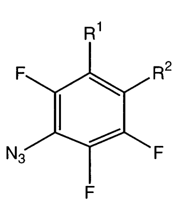


corresponding alcohol **11**[19a] with succinic anhydride (Scheme 1). The pyridyl ligand **2b** was prepared in four steps with an overall yield of about 30% as follows. First, the active esters **13a**,**b** were prepared by the reaction of mercaptoactetic acid with tetrahydropyranyl chloride and methyl chloroformate, respectively, followed by esterification of acid with *N*-hydroxysuccinimde (Scheme 2). Reaction of the ester **14**[19a] with 2-aminomethylpyridine (**15**) followed by hydrogenolysis of the α-Cbz group, condensation with the active ester **13a**, and deprotection with trifluoroacetic acid (TFA) resulted in **2b** (Scheme 2). Similarly, the imidazolyl ligand **3** was prepared in five steps with an overall yield of about 20% as follows: reaction of **14** with 2-aminomethyl-imidazole (**16**)[19a] followed by deprotection of the ϵ-*t*-BOC group, acylation with succinic anhydride, hydrogenolysis of the α-Cbz group, and condensation with the active ester **13a**. The histidyl ligand **4** was prepared in three steps with an overall yield of about 40% as follows: acylation of **17** with the active ester **13b**, esterification of the carboxyl group with *N*-hydroxysuccinimide, and condensation with histidine under alkaline conditions.

II. Synthesis of Perfluoroaryl Photoprobes. The azidoamide **18c** was prepared by esterification of the acid **18b** with *N*-hydroxysuccinimide followed by monoacylation of ethylenediamine with active ester **18a**. The azidoester **19c** and the azidonitrile **19d** were prepared from their corresponding tetrafluoro derivatives **19a** and **19b**[14,16] by nucleophilic aromatic substitution of fluorine at the position ortho to the methoxycarbonyl or cyano group in **19a** and **19b**, respectively, with excess ethylenediamine. The (λ_{max}) of chromogenic photoprobes **19c**,**d** exhibited bathochromic shifts to wavelengths greater than 330 nm, which permit photochemical conjugation at longer wavelengths. Photolysis at >320 nm is always desirable because it will minimize the perturbation of native structure of biomolecules under photolytic conditions.[16]



18a: R = —OCO
18b: R = -CO_2H
18c: R = -$CONH(CH_2)_2NH_2$

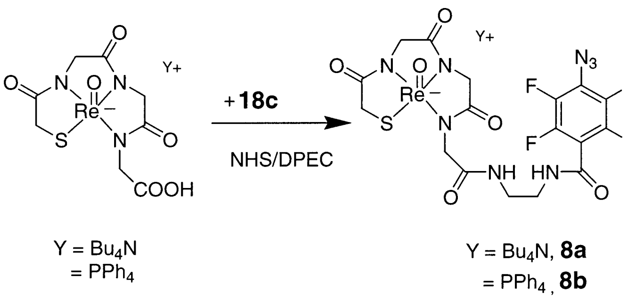


19a: R^1 = -CO_2Me, R^2 = -F
19b: R^1 = -CN, R^2 = -F
19c: R^1 = -CO_2Me, R^2 = -$NH(CH_2)_2NH_2$
19d: R^1 = -CN, R^2 = -$NH(CH_2)_2NH_2$

III. Synthesis of BFCA−Photoprobe Conjugates (BFPCAs). In general, N_3S- and N_2S_2-based ^{99m}Tc radiopharmaceuticals have been successfully used as noninvasive imaging probes in nuclear medicine.[3a] It is also known that hemithioacetals and hemithioketals are better protecting groups for sulfur compared to the benzoyl group with respect to metal complexation reactions.[19ac] In the present study, N_3S and N_2S_2 ligands **2a**,**b** **20a**,**b**, and **21** (Scheme 3) bearing tetrahydropyranyl, benzoyl, and ethoxyethyl sulfur protecting groups, respectively, were attached to photoprobes. BFPCAs **5a**,**d** and **6** were prepared in good yields by the condensation of the photoprobes **18c** with **20a**, **20b**, and **21** BFCAs, respectively, while, **5b**,**c**, **7a**, and **7b** were prepared by condensation of photoprobes **19c**,**d**, **18b**, and **18a** with **20a**, **20a**, **11**, and **2b** BFCAs, respectively, using a standard coupling procedure (HBTU) as shown in Scheme 3.

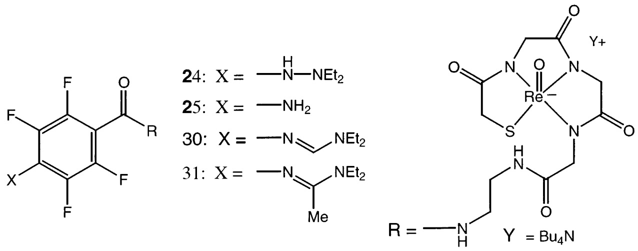
IV. Synthesis of Re−BFPCAs. Re complexes of MAG_3 (benzoyl-protected sulfur) were prepared using standard procedures with either tetrabutylammonium (TBA) or tetraphenylphosphonium (TPP) cations, respectively.[28] The complexes were treated with photoprobe **18c** in the presence of DPEC (1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride) and *N*-hydroxysuccinimide to give the TBA or TPP salts **8a** and **8b**, respectively, (Scheme 4). Re-photoprobe conjugates were characterized by ^{1}H, ^{31}P, ^{13}C, and ^{19}F NMR and MS. The mass spectrum of Re−BFPCA complex **8a**, for example, showed a pair of [M + 1] signals (m/z = 719.1 and 721.1) in a ratio of 1:2, characteristic of ^{185}Re and ^{187}Re isotopes. The infrared spectrum of **8a** shows the presence of strong bands at 2120 and 960 cm^{-1}, which are characteristic peaks for the N_3 and the Re(V)=O groups, respectively. These data clearly indicate that both the metal and the

SCHEME 4



azide group remained intact during the conjugation reaction under aqueous conditions; i.e., rhenium does not undergo oxidative decomposition with concomitant reduction of the azide moiety. This is particularly important since both azide group and metal in oxidation state V are required for conjugation of biomolecule and for imaging/thearpy studies, respectively. Re complex **8a** was extensively used to conduct photochemistry in model solvent and in labeling HSA and Fab fragments.

V. Photochemistry of Re−BFPCA Complex in Model Solvent. NH Insertion. It is important to verify the retention of favorable photochemical characteristics of Re-metalated photoprobes because photochemistry of metal complexes could be complicated due to demetalation followed by indiscriminate radical combination, oxidation, and reduction that occur after photolysis. In order for these agents to be useful in nuclear medicine, the conjugation should be mediated, in general through singlet nitrene rather than triplet nitrene.[16h] However, under certain circumstances, triplet nitrene can form a radical after abstracting a proton from the substrate followed by radical−radical combination leading to the insertion product, which is indistinguishable from singlet-mediated product.[15,16d] Successive abstraction of hydrogens by triplet nitrene will lead to amine **25**, which will not be useful for photobioconjugation. Photochemical reaction of Re complex **8a** in diethylamine solvent showed four products (**24**, **25**, **30**, and **31**) that were separated by column chromatography. Mass spectrum of Re complex **8a** after photolysis showed four molecular ion peaks of two sets each at *m*/*z* =764.3, 766.3 (major product), *m*/*z* = 776.3, 778.3 (minor product), *m*/*z* = 790.3, 792.3 (minor product) in addition to the triplet mediated noninsertion product at *m*/*z* = 693.2, 695.2 (see the Supporting Information). The major product was found to be the hydrazine derivative **24** originated from the singlet nitrene insertion into NH bond of diethylamine. The mass spectrum of **24**, isolated from the post-photolysis mixture, showed a clean set of *m*/*z* signals at δ 764.3 and 766.3 corresponding to 185 and 187 isotopes of Re. ^{19}F NMR of **24** showed a shift of the 3,5 fluorine signal to δ −157, a characteristic feature of singlet NH insertion in contrast to triplet product **25** in which the 3,5 fluorine signal shift to δ −164.[16e,h] The characterization of NH insertion product **24** to be the major product of photolysis signifies that the required intermolecular insertion properties by Re azides is retained in metalated BFPCA. Clearly, the Re metal complex remained stable during photolysis conditions.



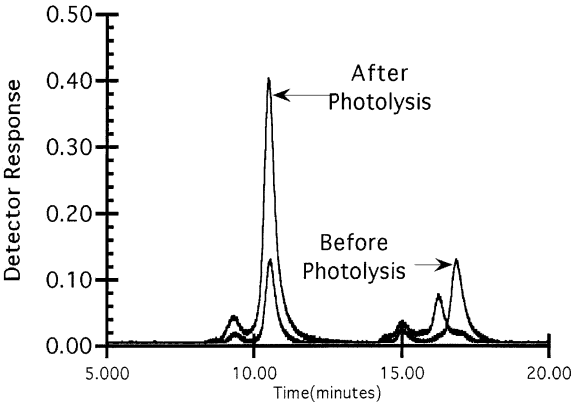


FIGURE 1.

Formation of minor products **25**, **30**, and **31** can be explained by the possible mechanism based on the similar product distribution by azides reported in the literature[34−36] and is outlined in Scheme 5. The triplet nitrene **23** abstracts hydrogen atoms from diethylamine to form the reduced product imine **26**, which rearranges to enamine **27**. Such dehydrogenation of dialkylamines is known to occur upon thermolysis or photolysis of azides in the presence of tertiary amines.[34] Subsequent equilibration to the imine **27** followed by the addition of diethylamine with an elimination of ethylamine yields diethylvinylamine (**28**). The vinylamine **28** undergoes 1,3-dipolar cycloaddition reaction with the azide **8a** to give the triazoline **29**. Compounds **30** and **31** are formed by the extrusion of either N$_2$ or CH$_2$N$_2$ molecules, respectively, from the triazoline **29**. The mechanism outlined here is consistent with the previous work on the thermal and photochemical reaction of azides in the presence of amines.[35,36] The successive abstraction of two hydrogens by triplet nitrene from diethylamine led to noninsertion product **25**. In essence, except compound **25**, all the photolysis products can be considered to be insertion products although, compounds **30** and **31** are routed through triplet nitrene, which makes the insertion efficiency of Re−BFPCA greater than 90% in diethylamine.

VI. Photochemical Conjugation of Re−BFPCA with HSA. A mixture of rhenium conjugate **8a** and HSA (3:1) was subjected to size-exclusion HPLC (Waters) prior to photolysis. Figure 1 shows a clear separation of the photoprobe **8a** (t_R = 16.8 min) and HSA (t_R = 10.4 min). However, after photolysis, it was observed that the intensity of the peak at t_R = 16.8 min (photoprobe) was reduced while the intensity of the peak at t_R = 10.4 min

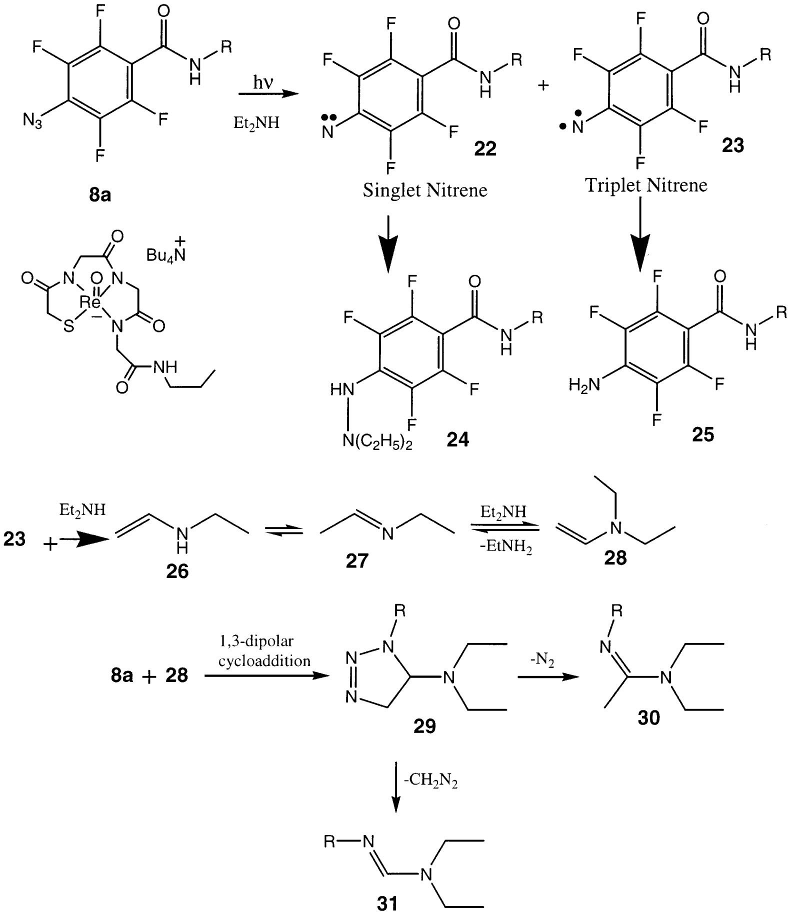
---

(34) Polanc, S.; Stanovnik, B.; Tisler, M. *J. Org. Chem.* **1976**, *41*, 3152.

(35) Polanc, S.; Stanovnik, B.; Tisler, M. *J. Heterocycl. Chem.* **1973**, *10*, 565.

(36) Polanc, S.; Versek, B.; Sek, B.; Tisler, M. *J. Org. Chem.* **1974**, *39*, 2143.

SCHEME 5



(protein) increased significantly (Figure 1). The additional product peak at about $t_R = 16.3$ min corresponds to the nonconjugated triplet-derived amine. Additional low intensity peaks at $t_R \sim 14-15$ min and at $t_R \sim 10$ min could be due to impurity in the Re−BFPCA preparation or HSA aggregate, respectively. The HPLC of the product after photolysis of **8a** in the absence of protein shows predominantly two peaks with $t_R = 16.3$ min and $t_R \sim 14-15$ min (Figure 2b), which coincides with triplet product and with the impurities, respectively, as stated above. In alternate studies, increasing the concentration of photoprobe with respect to HSA (e.g., 3, 5, and 10 times) resulted in the proportional increase in the intensity of the peak corresponding to HSA−photoprobe adduct (see the Supporting Information, Figure S1). Although it is clear from the present data and from our previous work[14c−e] that the photoprobe is covalently attached to the protein, the quantification of conjugation from the UV spectroscopic data alone is not possible and requires a radiomarker such as ^{99m}Tc.

VII. Photochemical Conjugation of Re−BFPCA with Fab Fragments. Attachment of photoprobes to Fab fragments was carried using the same methods as for HSA. Size-exclusion HPLC of a mixture of Fab fragments derived from human IgG (Accurate Chemical and Scientific Corp.) and the Re−BFPCA complex **8a** in an approximately 1:5 ratio is shown in Figure 2a. The retention times for the Fab fragment and complex **8a** are 12 and 16.3 min, respectively, under these chromatographic conditions. After photolysis, the intensity of the Fab peak increases significantly with a decrease in the intensity of the peak corresponding to the photoprobe **8a**. However, a greater abundance of triplet nitrene photoproducts are observed in this photolysis than those observed with HSA. It appears that that labeling is less efficient with F_{ab} compared to HSA. Again, the quantification of photoprbe attachment in the absence of a radiomarker is difficult. However, present protocol used for Re−BFPCAs can be extended to ^{99m}Tc−BFPCAs under tracer conditions for the quantification of efficiency of photoconjugation.

VII. Conclusions

In this study, we have demonstrated that: (a) covalent attachment of Re−BFPCA in HSA and Fab fragments can be achieved by a photochemical insertion method; (b) the intermolecular insertion efficiency of Re−BFPCA is high with no oxidation of Re metal or reduction of azide moiety in model solvent; (c) decomplexation of metal ion does not take place under photolytic conditions; and (d) synthesis of high specific activity prone photochemical probes has been achieved. In our earlier investigations on photochemical conjugation of the monoclonal antibodies[18] and bioactive drugs,[14a] we have shown that the immunoreactivity and biological activity respectively are retained in post photoconjugation. The combined results from the present work and previous studies clearly illustrate that photochemical conjugation is a useful technique for selectively delivering clinically useful metal

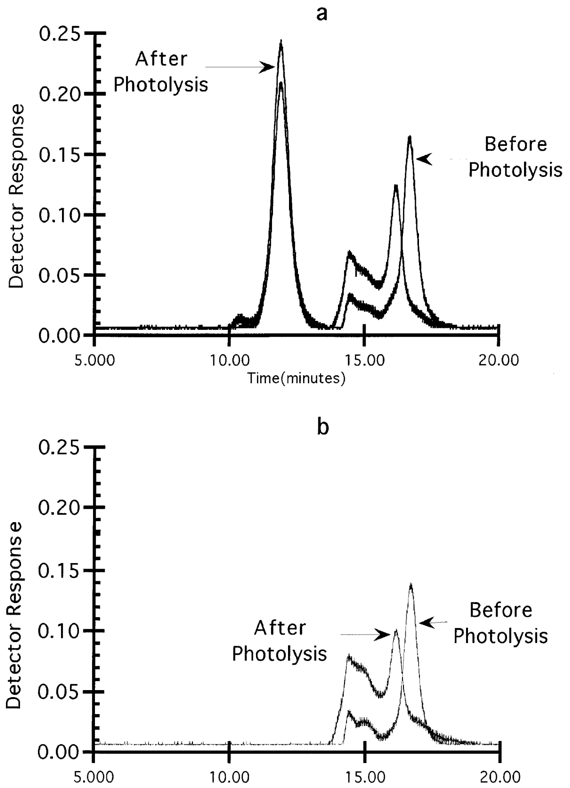


FIGURE 2.

ions to target tissues. We have also synthesized other types of ligands that are capable of forming metal complexes with high specific activity and potentially providing greater flexibility in tuning the lipophilicity of the eventual radiochemical conjugates. Finally, we have also characterized post-photolysis Re−BFPCA products in diethylamine and proposed a plausible mechanism of formation of minor products. Efforts to enhance the efficiency and quantification of insertion of BFPCAs into Fabs using ^{99m}Tc radiomarkers are in progress and will be published elsewhere.

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 in subdued light by wrapping the flasks with aluminum foil. Nuclear magnetic resonance spectra were recorded in $CDCl_3$, and chemical shifts are reported in ppm downfield from $SiMe_4$ for ^{1}H 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. In general, ^{13}C NMR spectra were taken at 75 MHz unless otherwise specified. In the ^{13}C NMR spectra of fluorinated compounds, signals due to C−F bonds are not reported due to the low intensity of carbon signals split by fluorines. ^{19}F NMR chemical shifts are reported with respect to $CFCl_3$ as an external standard. Elemental analyses for the new compounds were done by Oneida Research Services, Inc., New York. Chromatograms were obtained from Waters HPLC system fitted with a size-exclusion column (Biosil SEC 250, 5 μm, 100 A) and, in general, eluted with phosphate buffer (PBS, 0.02 M sodium phosphate, monobasic containing 0.05 M sodium sulfate, pH = 6.8 pumped at 1 mL/min.).

Photolysis was carried out with a 200 W high-pressure Hg lamp. The focused beam passed through a water filter before photolysis of soutions containing photoprobes. In general, organic azides are potentially explosive, and caution must be taken while preparing them. Metallic precursor Re gluconate was prepared as reported earlier.[20] Mass spectra were obtained with a mass spectrometer operated in positive or negative ion electrospray ionization modes. Samples were introduced using a syringe pump in a corresponding solvent at a flow rate 400 μL/min. The temperature of the heated capillary was 350 °C, electrospray voltage 4.5 kV (positive ions) or 3.0 kV (negative ions).

General Procedure for the Preparation of Active Esters 9a−d. A mixture of the desired heteroaromatic carboxylic acid (20 mmol), *N*-hydroxysuccinimide (20 mmol), and dicyclohexyl carbodiimide (20.5 mmol) in dry THF was stirred at ambient temperature for 12 h. The solid was filtered off and washed with dry THF, and the filtrate was evaporated in vacuo. The crude products were purified by recrystallization from methylene chloride/hexane. The analytical data for the specific compounds are given below.

Pyridyl Active Ester 9a. Yield: 3.2 g (71%). ^{1}H NMR ($CDCl_3$) δ: 8.82 (m, 1H), 8.22 (m, 1H), 7.92 (m, 1H), 7.60 (m, 1H), 2.85 (s, 4H). ^{13}C NMR ($CDCl_3$) δ: 168.75, 160.38, 150.44, 144.07, 137.22, 128.36, 126.55, 25.64. HRMS: calcd for $C_{10}H_8N_2O_4$ 220.0484, found 220.0479.

Pyrrole Active Ester 9b. Yield: 3.4 g (91%). ^{1}H NMR ($CDCl_3$) δ: 7.17 (m, 1H), 7.08 (m, 1H), 6.32 (m, 1H), 2.87 (s, 4H). ^{13}C NMR ($CDCl_3$) δ: 169.77, 155.71, 126.17, 119.43, 116.65, 111.50, 25.60. HRMS: calcd for $C_9H_8N_2O_4$ 208.0484, found 208.0491.

Thienyl Active Ester 9c. Yield: 3.7 g (84%). ^{1}H NMR ($CDCl_3$) δ: 8.03 (dd, J_1 = 1 Hz, J_2 = 3.2 Hz, 1H), 7.77 (dd, J_1 = 1 Hz, J_2 = 4.1 Hz, 1H), 7.2 (dd, J_1 = 3.2 Hz, J_2 = 4.1 Hz, 1H), 2.85 (s, 4H). ^{13}C NMR ($CDCl_3$) δ: 169.11, 157.27, 136.57, 135.65, 128.34, 126.84, 25.56. HRMS: calcd for $C_9H_7NO_4$ S 225.0096, found 225.0104.

Pyrazinyl Active Ester 9d. Yield: 3.4 g (76%). ^{1}H NMR ($CDCl_3$) δ: 9.39 (d, J = 1.2 Hz, 1H), 8.89 (d, J = 1.9 Hz, 1H), 8.82 (dd, J_1 = 1.2 Hz, J_2 = 1.9 Hz, 1H), 2.85 (s, 4H). ^{13}C NMR (DMSO) δ: 168.50, 159.46, 149.08, 146.97, 144.92, 139.99, 25.63. HRMS: calcd for $C_9H_7N_3O_4$ 221.0437, found 221.0441.

General Procedure for the Preparation of Heterocyclic Ligands 1a−d. A mixture of the active esters **9a−d** (11 mmol), 2,3-diaminopropanoic acid hydrochloride (5 mmol), and sodium bicarbonate (7.5 mmol) in acetonitrile/water (20:1) was stirred at ambient temperature for 16 h. The solvent was removed under reduced pressure, and the crude mixture was purified by chromatography over silica gel using acetonitrile/methanol (7:3). The analytical data for the specific compounds are given below.

Pyridyl Ligand 1a. Yield: 0.94 g (60%). ^{1}H NMR (DMSO) δ: 9.04−8.98 (m, 2H), 8.65−8.59 (m, 2H), 8.03−7.92 (m, 4H), 7.62−7.54 (m, 2H), 4.34 (dd, $J_1 = J_2$ = 6.3 Hz, 1H), 3.85−3.61 (m, 3H). ^{13}C NMR (125 MHz, DMSO) δ: 172.17, 163.93, 163.58, 149.67, 149.62, 148.54, 148.49, 137.86, 137.80, 126.67, 126.57, 121.85, 121.80, 53.37, 40.92. MS: m/z = 315.1 (M + H).

Pyrrolyl Ligand 1b. Yield: 0.80 g (56%). ^{1}H NMR (DMSO) δ: 11.54 (bs, 1H), 8.38 (t, J = 5 Hz, 1H), 8.21 (d, 1H), 6.82−6.85 (m, 2H), 6.73−6.76 (m, 2H), 6.07−6.02 (m, 2H), 4.36−4.44 (m, 1H), 3.71−3.53 (m, 2H). ^{13}C NMR (DMSO) δ: 172.68, 161.29, 161.09, 139.86, 139.81, 130.62, 128.71, 128.32, 127.83, 54.11. MS: m/z = 291.1 (M + H).

Thienyl Ligand 1c. Yield: 0.91 g (56%). ^{1}H NMR ($CDCl_3$) δ: 9.09 (t, 1H), 8.69 (d, 1H), 8.01 (d, 1H), 7.85 (d, 1H), 7.71 (t, 2H), 7.09 (m, 2H), 4.47 (m, 1H), 3.72 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 172.41, 161.51, 161.27, 139.77, 139.63, 130.81, 130.74, 128.87, 128.46, 127.90, 127.86, 107.8, 53.82. MS: m/z = 325.1 (M + H).

Pyrazinyl Ligand 1d. Yield: 1.3 g (84%). ^{1}H NMR ($CDCl_3$) δ: 9.17 (t, J = 4.2 Hz, 1H), 9.15 (d, J = 1.2 Hz, 2H), 8.86−8.84 (m, 3H), 8.73−8.70 (m, 2H), 4.17−4.11 (m, 1H), 3.91−

3.82 (m, 1H), 3.57−3.49 (m, 1H). ^{13}C NMR ($CDCl_3$) δ: 171.53, 162.56, 162.13, 147.59, 147.46, 144.50, 144.38, 143.36, 143.24, 53.21, 48.56, 41.24. MS: $m/z = 317.1$ (M + H).

Preparation of the Pyridyl Ligand 2a. A mixture of the alcohol **11**[19a] (1.10 g, 3.0 mmol), succinic anhydride (0.33 g, 3.1 mmol), and 4-dimethylaminopyridine (12 mg, 0.3 mmol) in dry acetonitrile was heated under reflux for 16 h. Upon slow cooling of the reaction mixture to ambient temperature, the product cyrstallized out as an off-white solid, which was collected by filtration, washed with cold acetonitrile, and dried in vacuo to obtain **2a** as an off-white solid. Yield: 860 mg (70%). ^1H NMR ($CDCl_3$) δ: 9.28 (bs, 1H), 8.45 (d, 1H), 7.92 (t, H-1H), 7.82−7.55 (m, 2H), 7.33 (dd, 1H), 7.27 (t, 1H), 4.78 (m, 1H), 4.55 (m, 1H), 4.50 (t, 2H), 4.25 (m, 1H), 4.15 (m, 1H), 3.95 (m, 1H), 3.41 (m, 1H), 3.35 (d, 2H), 3.20 (d, 1H), 2.15 (m, 4H), 2.15−1.51 (m, 6H). ^{13}C NMR ($CDCl_3$) δ: 176.9, 176.4, 172.6, 171.6, 170.5, 170.2, 156.6, 156.5, 149.1, 138.5, 123.4, 123.1, 106.6, 83.4, 83.3, 65.7, 65.1, 60.8, 50.4, 50.3, 43.8, 34.6, 31.8, 30.9, 29.8, 28.2, 25.0, 21.7, 21.4. MS: m/z 523.1 [M + H]. Anal. Calcd for $C_{21}H_{29}N_3O_7S$: C, 53.96; H, 6.21; N, 8.99; S, 6.85. Found: C, 54.08; H, 6.27; N, 9.00; S, 6.92.

Preparation of the Pyridyl Ligand 2b. A mixture of the *p*-nitrophenyl ester **14** (12.5 g, 25 mmol), and 2-aminomethylpyridine **15** (2.8 g, 26 mmol) in chloroform was stirred at ambient temperature for 16 h. The solution was repeatedly extracted with 10% NaOH solution until the organic layer was colorless. The organic layer was then washed with water, dried ($MgSO_4$), and filtered, and the filtrate was taken to dryness under reduced pressure to give the product as off-white foam. Yield: 9.5 g (75%). The material was used as such for the next step.

A solution of the pyridyl amide above (9.5 g, 25 mmol) in methanol was carefully treated with 10% Pd−C (2.0 g) and the mixture hydrogenated at 3.5 atm (50 psi) for 6 h. The reaction mixture was filtered through Celite, and the filtrate was evaporated in vacuo to give the amine as a pale yellow gum. Yield: 6.2 g (90%). The product was used as such for the next step.

A mixture of the amine above (4.03 g, 12 mmol) and *N*-succinimido-*S*-tetrahydropyranyl mercaptoacetate[19a] **13a** (3.20 g, 12.2 mmol) in dry acetonitrile (20 mL) was stirred at ambient temperature for 4 h. The reaction mixture was poured onto water (50 mL) and extracted with methylene chloride. The combined organic extracts were washed with water, dried ($MgSO_4$), and filtered, and the filtrate was taken to dryness under reduced pressure. The crude product was purified by column chromatography over silica gel using chloroform/methanol (95:5) as eluent. The desired fractions were pooled and evaporated under vacuo to give 2.6 g (50%) of the ligand **2b** as a waxy, pale tan solid. ^1H NMR ($CDCl_3$) δ: 8.47 (d, 1H), 7.60 (m, 1H), 7.50 (m, 1H), 7.38 (m, 1H), 7.21 (d, 1H), 7.15 (m, 1H), 4.60 (m, 2H), 4.48 (m, 3H), 3.62 (m, 1H), 3.50−3.18 (m, 3H), 3.02 (broad, 2H), 1.25 (s, 9H), 1.92−1.15 (m, 6H). ^{13}C NMR ($CDCl_3$) δ: 171.8, 170.0, 157.0, 156.7, 149.3, 137.0, 122.5, 121.9, 84.0, 83.6, 79.0, 66.2, 65.7, 53.2, 44.4, 40.0, 35.0, 34.6, 31.7, 31.0, 28.2, 25.0, 22.4, 21.6.

Preparation of the Imidazolyl Ligand 3. A mixture of the *p*-nitrophenyl ester **14** (5.0 g, 10 mmol), 2-aminomethylimidzole dihydrochloride **16** (1.70 g, 10 mmol), and triethylamine (2.20 g, 22 mmol) in acetonitrile was heated under reflux for 4 h. The reaction mixture was poured onto water, and the precipitate was collected by filtration and dried. Recrystallization from aqueous acetone afforded 3.2 g (60%) of amide as a colorless solid used as such for the next step.

The *t*-BOC derivative above (3.2 g) was treated with trifluoroacetic acid and kept at ambient temperature for 2 h, and thereafter, excess TFA was removed by evaporation in vacuo. The crude TFA salt of the product was treated with a solution of KOH (1.6 g in 3 mL of water) and purified by flash column chromatography over reversed-phase C-18 sorbent. The column was first eluted with water to remove salts and other polar impurities and thereafter eluted with water/methanol (3:1). The desired fractions were pooled and evaporated in vacuo to give the product amine as a pale green gum. Yield: 1.7 g (60%).

The ϵ-amino compound above (1.65 g, 5 mmol) and succinic anhydride (0.55 g, 5.5 mmol) in acetonitrile and isopropyl alcohol were heated under reflux for 2 h and stirred at ambient temperature for 16 h. The solvent was removed under reduced pressure, and the residue was redissolved in methanol and was carefully treated with 10% Pd−C (0.5 g). The mixture was hydrogenated at 3.5 atm (50 psi) for 4 h. The reaction mixture was filtered through Celite, and the filtrate was evaporated in vacuo to give the α-amino compound as a pale yellow gum. Yield: 6.2 g (90%). The product was used as such for the next step.

A mixture of the amine above (1.55 g, 5 mmol) and *N*-succinimido-*S*-tetrahydropyranylmercaptoacetate **13a** (1.31 g, 5 mmol) in dry acetonitrile was stirred at ambient temperature for 16 h. The solvent was evaporated in vacuo, and the crude product was purified by flash column chromatography over reversed-phase C-18 sorbent. The column was first eluted with water to remove *N*-hydroxysuccinimide and other polar impurities and thereafter eluted with water/methanol (3:1). The desired fractions were pooled and evaporated in vacuo to give the desired ligand **3** as an off-white amorphous solid. Yield: 1.4 g (50%). ^1H NMR (D_2O) δ: 6.78 (s, 2H), 4.17 (dd, 2H), 3.78 (m, 1H), 3.45 (m, 1H), 3.10−2.80 (m, 4H), 2.65 (t, 2H), 2.15 (m, 2H), 1.95 (m, 2H), 1.42−1.15 (m, 6H). ^{13}C NMR (D_2O) δ: 181.3, 177.5, 177.2, 175.6, 145.7, 121.1, 84.9, 84.8, 79.0, 68.0, 56.0, 40.7, 36.3, 35.3, 33.2, 33.0, 32.5. 32.1, 29.6, 26.5, 24.0, 23.2.

Preparation of the Active Ester 13b. A mixture of mercaptoacetic acid (9.20 g, 0.10 mol) and triethylamine (22.22 g, 0.22 mol) in acetonitrile/water was stirred and cooled to 0 °C in an ice−salt bath. Thereafter, methyl chloroformate (10.40 g, 0.11 mol) was added dropwise via pressure-equalized addition funnel at such a rate that the temperature of the solution did not exceed 15 °C. After the addition, the reaction mixture was stirred at ambient temperature for 4 h. The reaction mixture was concentrated to about half the volume, treated with concentrated HCl, and extracted with ethyl acetate. The combined organic extracts were washed with brine, dried (Na_2SO_4), and filtered, and the filtrate was evaporated in vacuo. The crude product was purified by distillation under reduced pressure (100−105 °C, 0.2 Torr) to give 13.5 g of colorless liquid that solidified upon standing.

A mixture of *S*-methoxycarbonylmercaptoacetic acid above (1.18 g, 10 mmol) -hydroxysuccinimide (1.15 g, 10 mol) and dicylohexyl carbodiimide (DCC) (2.06 g, 10 mol) in dry acetonitrile was stirred at ambient temperature for 16 h. The reaction mixture was filtered to remove dicyclohexyl urea (DCU) and the filtrate evaporated in vacuo. The residue was triturated with ether and the colorless solid **13b**, which was collected by filtration and dried. The material was used as such for the next step.

Preparation of the Histidyl Ligand 4. A mixture of the active ester **13b** above (2.47 g, 10 mmol), *N*-ϵ-BOC lysine **17** (3.42 g, 10 mmol), and triethylamine (2.02 g, 20 mmol) in dry acetonitrile was stirred at ambient temperature for 16 h. The reaction mixture was poured onto water, treated with concentrated HCl, and extracted with methylene chloride. The combined organic layers were washed with brine, dried (Na_2SO_4), and filtered, and the filtrate was evaporated in vacuo to give 3.3 g of pale brown gum, which was used as such for the next step.

A mixture of the above acid (3.78 g, 10 mmol), *N*-hydroxysuccinimide (1.15 g, 10 mol), and dicylohexylcarbodiimide (DCC) (2.06 g, 10 mol) in dry acetonitrile was stirred at ambient temperature for 16 h. The reaction mixture was filtered to remove dicyclohexyl urea (DCU) and the filtrate evaporated in vacuo. The residue was triturated with ether and the colorless solid, which was collected by filtration and dried. The material was used as such for the next step.

The active ester above (1.18 g, 2.5 mmol) was dissolved in isopropyl alcohol and treated with a solution of histidine (0.40 g, 2.6 mmol) in 2.5 mL of 1.0 N NaOH. After the addition, the mixture was stirred at ambient temperature for 48 h. The reaction mixture was poured onto acetone. The solid was collected by filtration, washed with acetone, and dried to obtain histidyl ligand **4**. Yield: 1.4 g (50%). ^{1}H NMR (D_2O) δ: 7.95 (s, 1H), 7.00 (d, 1H), 4.45 (m, 1H), 4.38 (m, 1H), 3.80 (s, 3H), 3.20−2.80 (m, 4H), 1.75−1.50 (m, 6H), 1.40 (s, 9H).

Preparation of Azide 18c. A solution of perfluoroazidosuccinimide **18a** (3.5 g, 10.542 mmol) in acetonitrile was added dropwise over a period of 15 min into an excess of ethylenediamine (11.123 g, 185.08 mmol) in acetonitrile. After the mixture was stirred for an additional 45 min, a white precipitate was formed that was filtered and washed with acetonitrile The solution was diluted with equal amounts of water and extracted with chloroform. The combined organics were washed with water and brine, dried over $MgSO_4$, and evaporated in a drying dish overnight. A pale yellow colored solid amine **18c** (1.2 g, 41%) was obtained. ^{1}H NMR ($CDCl_3$) δ: 6.4 (b, 1H), 3.6 (m, 2H), 3.2 (2H), 2.9 (s, 2H). MS: m/z = 277.9 (M + H).^{13}C NMR (DMSO) δ: 168.61, 160.25, 25.63.

Preparation of the Azide 19d. A solution of the nitrile **19b** (3.5 g, 16.196 mmol) in methylene chloride was added dropwise over a period of 30 min to ethylenediamine (4.87 g, 80.989 mmol) also taken in methylene chloride. The mixture was transferred to separatory funnel, extracted with CH_2Cl_2, stirred for 2 h, washed with water and brine, and dried over $MgSO_4$. The solvent was removed under vacuum to give compound **19d** (3.675 g, 88.57% yield). ^{1}H NMR ($CDCl_3$) δ: 5.12 (s, 1H), 3.6−3.47 (m, 2H), 3.01−2.93 (m, 2H), 2.83 (s, 2H). ^{13}C NMR (DMSO)_δ, 206.61, 125.72, 112.75, 51.15, 46.28, ^{19}F NMR ($CDCl_3$) δ: −135.24, −147.78, −161.67. HRMS: calcd for $C_9H_7N_6F_3$ 256.0684, found 256.0673. UV (CH_3OH): λ_{max} = 330 nm.

Preparation of the MAG$_3$ Ligand 21. A mixture of EOE-succinimide[19d] (4.00 g, 15.32 mmol) in acetonitrile/water (20:1, 75 mL), triglycine (2.635 g, 13.93 mmol), and sodium bicarbonate (1.287 g, 15.32 mmol) was stirred overnight. The solvent was removed under reduced pressure, and the crude mixture was flash chromatographed (AcCN/MeOH, 70:30) to give the acid **21** (4.16 g, 75.4% yield). ^{1}H NMR (DMSO) δ: 8.24−8.17 (m, 2H), 7.93 (t, J = 5.0 Hz, 1H), 4.79 (q, J = 6.3 Hz, 1H), 3.75−3.71 (m, 4H), 3.65−3.60 (m, 3H), 3.44−3.33 (m, 2H), 3.23 (s, 2H), 1.42 (d, J = 6.3 Hz, 3H), 1.08 (t, J = 7.0 Hz, 3H). ^{13}C NMR (DMSO) δ: 171.10, 169.40, 168.98, 167.80, 80.77, 62.07, 42.25, 42.12, 40.33, 31.45, 22.48, 14.84. MS: m/z = 358.1 (M + Na).

Preparation of the Photoprobe 5a. The MAG$_3$ ligand **20a** (0.5 g, 1.36 mmol) was taken in dry DMF (25 mL). HBTU (0.56 g, 1.36 mmol) and N-methylmorpholine (0.53 g, 5.25 mmol) were added followed by photoprobe amine **18c** (0.29 g, 1.05 mmol) also taken in DMF. The mixture was stirred for 4 h and filtered, and solid was thoroughly washed with methanol to obtain **5a** (Yield 0.485 g, 74%). ^{1}H NMR (DMSO) δ: 8.89 (t, 1H), 8.45 (t, 1H), 8.17 (t, 1H), 8.10 (t, 1H), 7.94−7.53 (7H), 3.87 (s, 2H), 3.84−3.66 (8H), 3.4−0.3.2 (4H). ^{19}F NMR (DMSO) δ: −142.43, −151.25.

Preparation of Photoprobe 5b. The MAG$_3$ ligand **20a** (1.975 g, 5.38 mmol) in dry DMF (50 mL) was mixed with amine **19c** (1.556 g, 5.38 mmol), HBTU (2.55 g, 6.725 mmol), and N-methylmorpholine (2.72 g, 26.9 mmol). The mixture was allowed to stir at room temperature under inert atmosphere (N_2) for 16 h. The solution was filtered, and filtrate was poured into ether. The precipitated solid was filtered, washed with methanol, and dried to obtain compound **5b**. Yield: 1.96 g, 57%. ^{1}H NMR (DMSO) δ: 8.46 (t, J = 5.6 Hz, 1H), 8.19 (t, J = 5.6 Hz, 1H), 8.07 (t, J = 5.6 Hz, 1H), 7.94−7.90 (m, 2H), 7.85 (t, J = 5.4 Hz, 1H), 7.73−7.67 (m, 1H), 7.58−7.52 (m, 2H), 6.53 (s, 1H), 3.87 (s, 2H), 3.85 (s, 3H), 3.79−3.73 (m, 4H), 3.65 (d, J = 5.7 Hz, 2H), 3.3−3.20 (m, 4H). ^{13}C NMR (DMSO) δ: 190.27, 169.15, 169.10, 169.00, 167.27, 164.40, 135.90, 134.03, 129.09, 126.84, 52.79, 45.32, 45.17, 42.50, 42.17, 41.94, 32.43. ^{19}F NMR (DMSO) δ: −139.20, −143.90, −162.58. MS: m/z = 661.1 (M + Na).

Preparation of the Photoprobe 5c. The MAG$_3$ ligand **20a** (0.537 g, 1.463 mmol) in dry DMF (35 mL) and amine **19d** (0.300 g, 1.171 mmol) were mixed with HBTU (0.555 g, 1.463 mmol) and N-methylmorpholine (0.592 g, 5.855 mmol) followed by stirring at room temperature under inert atmosphere (N_2) for 16 h. The solution was filtered and poured into ether. The precipitated solid was filtered, washed with methanol, and dried to obtain compound **5c** (0.485 g, 68.4%). ^{1}H NMR (DMSO) δ: 8.48 (t, J = 5.6 Hz, 1H), 8.22 (t, J = 5.6 Hz, 1H), 8.12 (t, J = 5.8 Hz, 1H), 7.93−7.88 (m, 3H), 7.71−7.69 (m, 1H), 7.57−7.54 (m, 2H), 6.5 (s, 1H), 3.87 (s, 2H), 3.77 (d, J = 5.6 Hz, 2H), 3.74 (d, J = 5.6 Hz, 2H), 3.67−3.65 (m, 2H), 3.53−3.45 (m, 2H), 3.32−3.25 (m, 2H). ^{13}C NMR (DMSO) δ: 190.28, 169.42, 169.20, 169.06, 167.28, 135.88, 134.08, 129.13, 126.86, 44.20, 42.48, 42.17, 41.96, 38.88, 32.45. ^{19}F NMR (DMSO) δ: −137.84, −145.42, −163.18. MS: m/z = 628.1 (M + Na).

Preparation of the Photoprobe 5d. Mono-EOE-protected N_3S acid **20b** (1.8 g, 5.367 mmol), HBTU (2.036 g, 5.368 mmol), and N-methylmorpholine (3.132 g, 30.96 mmol) were mixed in acetonitrile, followed by the addition of photoprobe amine **18c** (1.143 g, 4.128 mmol). The mixture was stirred overnight and filtered. The solid was thoroughly washed with methanol and dried to give compound **5d** (1.25 g, 39.2% yield). ^{1}H NMR (DMSO) δ: 8.92 (t, J = 5.2 Hz, 1H), 8.21−8.1 (m, 3H), 7.9 (m, 1H), 4.79 (q, J = 6.2 Hz, 1H), 3.75−3.57 (m, 8H), 1.42 (d, J = 6.2 Hz, 3H), 1.08 (t, J = 7.0 Hz, 3H). ^{13}C NMR (DMSO) δ: 169.57, 169.24, 169.08, 168.98, 157.08, 80.78, 62,12, 42.34, 42.09, 37.95, 31.38, 22.49, 14.88. ^{19}F NMR (DMSO) δ: −142.46 (m, 2F) −151.25 (m, 2F). MS: m/z = 617.4 (M + Na).

Preparation of N_2S_2 Ligand 21. EOE succinimide[19d] (4.086 g, 15.65 mmol) was taken in acetonitrile/water (20:1, 75 mL). 2,3-Diaminopropanoic acid hydrochloride (1.0 g, 7.114 mmol) and sodium bicarbonate (0.896 g, 10.67 mmol) were added, and the mixture was stirred for 12 h. The solvent was removed under reduced pressure, and the crude mixture was flash chromatographed over silica gel using AcCN/MeOH (70:30) as eluent to obtain the pure product **21** (1.97 g, 70%). ^{1}H NMR (DMSO) δ: 8.09(m, 1H), 8.07(m, 1H), 4.81−4.72(m, 2H), 4.08 (dd, $J_1 = J_2$ = 6.6 Hz, 1H), 3.69−3.56 (m, 2H), 3.45−3.32 (m, 6H), 2.57 (s, 2H), 1.43−1.39 (m, 6H), 1.08 (t, J = 7 Hz, 6H). ^{13}C NMR (DMSO) δ: 171.94, 169.07, 168.71, 80.81, 80.74, 62.5, 62.24, 62.15, 62.10, 53.03, 48.54, 31.58, 22.62, 22.49, 14.88. MS: m/z = 419.1 (M + Na).

Preparation of the Photoprobe 6. The EOE-protected N_2S_2 acid **21** (0.940 g, 2.373 mmol) in acetonitrile (50 mL) was mixed with HBTU (0.900 g, 2.373 mmol) and N-methylmorpholine (0.923 g, 1.003 mL, 9.13 mmol) before the photoprobe amine **18c** (0.506 g, 1.862 mmol) was added, and the mixture was stirred overnight. The solvent was removed under reduced pressure, and the crude mixture was flash chromatographed (CH_2Cl_2/MeOH, 97:3) to obtain the pure compound **6** (0.490 g, 41% yield). ^{1}H NMR ($CDCl_3$) δ: 7.87 (t, J = 5.5 Hz, 1H), 7.81 (t, J = 5.9 Hz, 1H), 7.49 (m, 2H), 4.79 (m, 2H), 4.48 (m, 1H), 3.75−3.41 (m, 10H), 3.28−3.20 (m, 4H), 1.52 (m, 6H), 1.17 (m, 6H). ^{13}C NMR $CDCl_3$) δ: 172.1, 169.62, 169.19, 168.27, 80.86, 62.16, 42.93, 42.36, 42.14, 31.50, 22.56, 14.94. ^{19}F NMR ($CDCl_3$) δ: −141.23 (m), −150.88 (m). MS: m/z = 678.4 (M + Na).

Preparation of the Photoprobe 7a. The alcohol **11**[19a] (0.300 g, 0.816 mmol) in dry THF (30 mL) with perfluoro aryl photoprobe acid **18b** prepared earlier[15a] (0.192 g, 0.816 mmol) was added along with DCC (0.185 g, 0.897 mmol), and the mixture was stirred at room temperature under inert atmosphere (N_2) for 24 h. The solution was filtered, the solvent removed and flash chromatographed over silica gel using CH_2Cl_2/MeOH (98:2) as eluent to obtain product **7a** (0.189 g, 39.6%). ^{1}H NMR ($CDCl_3$) δ: 8.49 (d, J = 4.6 Hz, 1H), 7.7−7.61 (m, 2H), 7.46−7.41 (m, 1H), 7.27−7.16 (m, 2H), 4.78−4.68 (m, 2H), 4.56−4.43 (m, 4H), 4.04−3.93 (m, 1H), 3.53−

3.40 (m, 1H), 3.82 (dd, $J_1 = 16$ Hz, $J_2 = 3$ Hz, 2H), 2.42−2.34 (m, 1H), 2.25−2.13 (m, 1H), 1.99−1.52 (m, 6H). ^{13}C NMR ($CDCl_3$) δ: 170.51, 169.82, 156.33, 156.06, 149.06, 136.72, 121.65, 121.6, 84.19, 83.84, 66.71, 63.13, 50.72, 44.59, 35.31, 34.94, 31.19, 30.97, 25.14, 22.30. ^{19}F NMR (DMSO) δ: −138.35, −150.86. MS: $m/z = 585.5$ (M + H).

Preparation of the Photoprobe 7b. The amine **2b** (0.075 g, 0.19 mmol) was taken in acetonitrile (5.0 mL). Perfluoroaryl-succinamide[14g] **18a** (0.07 g, 0.209 mmol) was added, and the mixture was stirred for 4 h. The solvent was removed under reduced pressure, and the crude mixture was flash chromatographed over silica gel to obtain compound **7b** (0.089 g, 76.5% yield). ^{1}H NMR ($CDCl_3$) δ: 8.50 (d, $J = 4.6$ Hz, 1H), 7.7−7.61 (m, 1H), 7.58−7.49 (m, 2H), 7.29−7.19 (m, 2H), 6.71−6.61 (m, 1H), 4.79−4.54 (m, 1H), 4.54−4.52 (m, 3H), 4.04−3.96 (m, 1H), 3.48−3.30 (m, 3H), 3.35 (d, 1H), 3.27−3.19 (m, 1H), 1.9−1.2 (m, 12H). ^{13}C NMR ($CDCl_3$) δ: 173.01, 171.51, 170.0, 157.79, 156.23, 148.60, 137.41, 122.61, 122.27, 84.02, 83.84, 66.25, 65.70, 52.9, 50.72, 44.32, 39.71, 35.22, 34.94, 31.71, 31.16, 28.00, 25.44, 25.21, 22.31. ^{19}F NMR (DMSO) δ: −138.35, −150.86. MS: $m/z = 612.4$ (M + H).

Synthesis of Re−MAG$_3$ Photoprobes 8a. The TBA (tetrabutylammonium) salt[22a] of ReOMAG$_3$ (200 mg, 028 mmol) was mixed with photoprobe **18c** (88 mg, 0.32 mmol), NHS (36 mg, 0.32 mmol), and DPEC (1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, 71 mg, 0.37 mmol) in anhydrous CH_2Cl_2, and the solution was stirred under nitrogen until HPLC indicated complete reaction of photoprobe **18c** (∼7 h). The solution was filtered, washed with H_2O twice, dried, and evaporated to give 189 mg (62%) of crude product. Flash chromatography using 3% MeOH in CH_2Cl_2 as eluent yielded two fractions that had the same HPLC retention time and spectroscopic properties. Both fractions were collected and evaporated to obtain **8a** as the tetrabutylammonium salt (21% yield), which was used for bioconjugation. ^{1}H NMR ($CDCl_3$) δ: 5.21−3.74 (AB patten for CH_2 protons, 8H), 3.61 (m, 2H), 3.36 (m, 2H), TBA peaks, 3.15 (m, 8H), 1.61−0.98 (28 H). ^{19}F NMR ($CDCl_3$) δ: −140.75 (m, 2F), −154.68 (m, 2F). MS: m/z =719.1 and 721.1 (M^- ion corresponds to 185 and 187 Re isotopes).

The tetraphenylphosphonium (TPP) salt[22a] of ReOMAG$_3$ (200 mg, 0.25 mmol) was dissolved in 10 mL of anhydrous CH_2Cl_2 and was mixed with photoprobe 2 (69 mg, 0.25 mmol), NHS (29 mg, 0.25 mmol), and DPEC (1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, 57 mg, 0.30 mmol), and the solution was stirred under nitrogen until HPLC indicated complete reaction of photoprobe **18c**. The solution was filtered and washed with water two times. The organic layer was separated, dried, and evaporated to obtain the crude product **8b** as a tetraphenylphosponium salt (64% yield). Flash chromatography of product **8b** using 3% MeOH in CH_2Cl_2 as eluent yielded pure product (yield 21%**)**. ^{1}H NMR ($CDCl_3$) δ: 7.61−7.31 (20 H, PPh_4), 5.31−3.85 (AB patten for CH_2 protons, 8H), 3.65 (m, 2H), 3.38 (m, 2H), ^{19}F NMR ($CDCl_3$) δ −141.25 (m,2F), −154.32 (m, 2F). MS: m/z =719.1 and 721.1 (M^- ion).

Photochemistry of 8a in Diethylamine. A solution of Re complex-photoprobe **8a** in a mixture of diethylamine and CH_2Cl_2 was exposed for 10 min to a light from a 200 W high-pressure Hg lamp filtered to remove wavelengths below 310 nm. The mass spectrum of the mixture showed four products. The solution was evaporated and flash chromatographed using 3% MeOH in CH_2Cl_2 to separate NH insertion product hydrazine **24** as a major product and 3 other minor **products 25**, **31**, and **32**.

Analytical Data. Compound 24. MS: $m/z = 764.3$, 766.3. ^{19}F NMR ($CDCl_3$) δ: −143.4, −157.2.

Compound 25. MS: $m/z = 693.2$, 695.2. ^{19}F NMR ($CDCl_3$) δ: −144.8, −164.2.

Compound 31. MS: $m/z = 790.3$, 792.3.

Compound 32. MS: $m/z = 776.3$, 778.3.

Acknowledgment. The work was supported by funds provided by NIH Grant No. CA 72421 and American Heart Association Grant No. AHA 99142621. NMR data were collected on instruments purchased with funds from NSF Grant Nos. 8908304 and 9221835. We appreciate the help of Dr. Guo of the NMR facility for help with the decoupling experiments.

Supporting Information Available: ^{1}H and ^{13}C NMR of compounds **1b**−**d**, **5b**−**d**, **6**, **7a**,**b**, **9a**−**d**, **19d**, **20b**, and **21** and MS of **24**, **25**, **30**, and **31**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO010782U