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New Reagents for Photoaffinity Labeling: Synthesis and Photolysis of Functionalized Perfluorophenyl Azides

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Functionalized perfluorophenyl azides (PFPA) **2–6**, **8–12**, **14–19**, and **21–29** were synthesized, allowing the attachment of PFPA to other molecules for application as photolabels. Two bioactive molecules were modified by a PFPA as potential photoaffinity labeling reagents. Photolysis of two representative members was investigated. Photolysis of azide **4** in cyclohexane gave 57% of CH insertion product. When photolysis was carried out in the presence of diethylamine as a trapping reagent, it gave 65% of NH insertion product, and no ring expansion product was found. The nitro azide **19** showed a shoulder absorption in the longer wavelength compared to azide **4**. Azide **19** gave less CH insertion and more aniline products compared to azide **4** when photolyzed in toluene, suggesting that the nitro group accelerates intersystem crossing of the singlet nitrene or the singlet excited azide. Collectively, our results demonstrate that the functionalized PFPA investigated are much better in undergoing CH insertion than their nonfluorinated analogues and suggest that they may constitute an improved series of photolabeling reagents.

Introduction

Photoaffinity labeling (PAL) has been widely used in biochemistry and molecular biology to study the proximity of components within biological systems.[1] A photoaffinity labeling reagent is a biological ligand modified with a photoactive moiety such as a diazo or azido group, which serves as a precursor for the highly reactive intermediate carbene or nitrene generated upon photolysis. The success of the PAL technique depends on the formation of a covalent bond between the photolabel and the targeted biopolymer. CH insertion by the carbene or nitrene is the most desirable pathway for establishing the stable covalent linkage and has stimulated efforts to develop new reagents which undergo CH insertion efficiently.[2]

Aryl azides are among the most widely applied photolabel reagents.[3] However, photolysis of phenyl azide in hydrocarbon solvents at room temperature gives almost no intermolecular CH insertion products.[4] The singlet phenyl nitrene generated instead rapidly undergoes ring expansion to a dehydroazepine,[5,6] which tends to be intercepted by a nucleophile such as an amine or undergoes polymerization to form tar[7] in the absence of a nucleophile. Formation of the less reactive (hence longer lifetime) dehydroazepine electrophilic intermediate can lead to decreased efficiency of site-directed labeling and increased nonspecific labeling.

Perfluorophenyl azides (PFPA) showed promise as an improved series of photolabels. Banks reported in 1972 that thermal decomposition of 4-azidotetrafluoropyridine in cyclohexane gave 45% yield of CH insertion product.[8] Later Dunkin discovered that pentafluorophenyl nitrene did not undergo ring expansion photochemically in N_2 or Ar matrices at 12 K.[9] Bayley and Staros pointed out in 1984 that PFPA might constitute an unexplored avenue for development of new photolabel reagents.[3] Platz reported recently that photolysis of pentafluorophenyl azide at 25 °C gave 52% yield of CH insertion in toluene[10] and 28% in cyclohexane.[11] The availability of ^{19}F NMR as a convenient and unique probe[12] for monitoring the photolysis products of PFPA is also a potential advantage.

There were only a few known functionalized PFPA such as CN,[13] CF_3,[14] and NO_2[15] before we began our work.[16,17] With applications of PFPA for PAL studies beginning to appear,[18] it is important to determine whether Dunkin's low-temperature observation can be extended to room temperature conditions because ring expansion of pentafluorophenyl nitrene during pyrolysis at 300 °C of pentafluorophenyl azide has been reported.[19] Since 2,6-difluorophenyl azide gave much less C-H insertion products (17%) than pentafluorophenyl azide when photolyzed under identical conditions,[10] it is also important to determine whether functionalized perfluorophenyl azides behave photochemically similar to pentafluorophenyl azide. In our preliminary papers[16,17] we have reported the synthesis of several new PFPA photolables bearing chemically reactive groups. Herein we report the details of the synthesis of those PFPA as well as several new reagents

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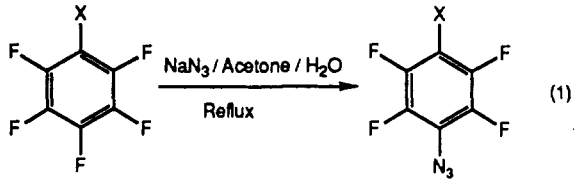
and the modification of two bioactive molecules with a PFPA. We also report the photolysis of two representative members of these new photolable reagents, namely azides 4 and 19.

Results and Discussion

A. Synthesis and Application of Functionalized Perfluorophenyl Azides. It is known that pentafluorophenyl compounds containing electron-withdrawing groups such as pentafluorobenzonitrile[13] and octafluorotoluene[14] react with many nucleophilic reagents, including sodium azide, to give 1,4-disubstituted compounds regioselectively. Thus, the para-functionalized perfluorophenyl azides 1–6 were synthesized from the corresponding pentafluorophenyl compounds in good yield (eq 1). These



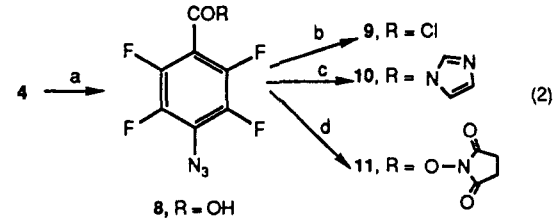
(1)

1, X = CN 4, X = CO_2Me
2, X = $CONH_2$ 5, X = COMe
3, X = CHO 6, X = NO_2

functional groups were selected because the starting materials are readily available. It was found that the perfluorophenyl azide moiety is stable under a variety of reaction conditions; therefore we were able to transform the functional groups in azides 1–5 into other functional groups. ^{19}F NMR spectra of compounds 1–5 show the expected AA'XX' pattern[20] with practically no ortho isomer being detected in the crude reaction mixture. Additionally, aldehyde 3 shows that both F_2, F_6 and F_3, F_5 have F–H coupling with the aldehyde proton, while azide 5 shows that only F_2, F_6 has F–H coupling with the methyl protons. The ^{19}F NMR spectral data for the 1,4-disubstituted compounds are collected in the supplementary material.

4-Azidotetrafluorobenzamide (2) was obtained as a white solid in 83% yield from pentafluorobenzamide. Basic hydrolysis (H_2O_2/NaOH/CH_2Cl_2/(n-$C_4H_9)_4NHSO_4$, 25 °C)[21] of nitrile 1[13] also gave azide 2 but only in 18% yield, possibly due to decomposition of the azide moiety. 4-Azidotetrafluorobenzaldehyde (3) was obtained as a colorless solid in 95% yield from pentafluorobenzaldehyde. Below we will show the attachment of azide 3 to another molecule (33 → 34) by a reductive amination reaction. Methyl 4-azidotetrafluorobenzoate (4) was obtained as a colorless solid in 87% yield from methyl pentafluorobenzoate (7).[22] Ester 7 was prepared as a liquid from pentafluorobenzoic acid in 97% yield. 4'-Azido-2',3',5',6'-tetrafluoroacetophenone (5) was obtained as a colorless liquid in 80% yield from 2',3',4',5',6'-pentafluoroacetophenone. 4-Azidotetrafluoronitrobenzene (6)[15] was obtained as a pale yellow liquid from pentafluoronitrobenzene. ^{19}F NMR of the reaction mixture showed the presence of <5% of the ortho isomer as reported.[15]

Basic hydrolysis (NaOH/MeOH/H_2O) of ester 4 gave 4-azidotetrafluorobenzoic acid (8) (eq 2) as a colorless solid in 98% yield. Acid 8 also was obtained from hydrolysis of amide 2 (Na_2O_2/water, 50 °C)[23] in 64% yield. 4-Azidotetrafluorobenzoyl chloride (9) was obtained as a pale

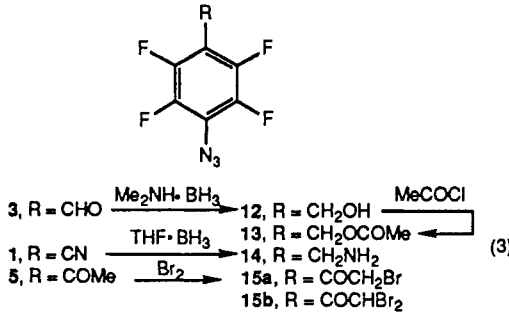


(2)

a) 1. NaOH / MeOH / H_2O, 2. HCl. b) $SOCl_2$.
c) CDI. d) NHS / DCC.

yellow liquid in 70% yield by refluxing acid 8 in excess thionyl chloride in dry CH_2Cl_2. The acylimidazole 10 was obtained as a colorless solid in 70% yield by reaction of acid 8 with 1,1-carbonyldiimidazole (CDI)[24] in dry THF. Azides 9 and 10 should find application as acylation reagents with available hydroxy or amine groups for attachment of PFPA to other molecules. *N*-Hydroxysuccinimidyl 4-azidotetrafluorobenzoate (11) was obtained as a solid in 99% yield by reaction of acid 8 with *N*-hydroxysuccinimide (NHS) and dicyclohexylcarbodiimide (DCC).[25] The advantage of NHS esters over other acylation reagents is that NHS esters react preferentially with amino groups and have been widely used for modification of proteins under aqueous conditions.[26] Therefore NHS ester 11 should find application as a bifunctional crosslinking reagent under the conditions similar to its nonfluorinated analogue.[27]

Selective reduction of aldehyde 3 was accomplished with $Me_2NH{\cdot}BH_3$[28] to give 4-azidotetrafluorobenzyl alcohol (12) as a colorless solid in 98% yield. Alcohol 12 was readily acetylated by acetyl chloride to produce ester 13 (eq 3),



(3)

suggesting that alcohol 12 could be attached to other molecules through acylation with an available carboxyl group. Alcohol 12 also might prove useful as a starting point for the synthesis of a photoactive phenylalanine.[29] Reduction of nitrile 1 by THF·BH_3[30] gave 4-azidotetrafluorobenzylamine (14) as a liquid in 48% yield. One of the possible advantages of amine 14 over alcohol 12 is that amines could react with an available NHS ester to form an amide in the presence of hydroxy groups.[31] Bromination of azide 5 by Br_2 in ether[32] gave the phenacyl bromide 15a as a liquid in 71% yield together with dibromide 15b. Since the nonfluorinated analogue of 15a

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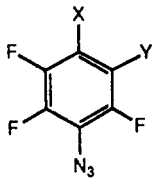
Table I. UV Spectra (EtOH; λ, nm) of the Substituted Perfluorophenyl Azides



compd	X	Y	λ_{max}	log ϵ_{max}	log ϵ_{300}
2	$CONH_2$	F	255	4.30	2.67
3	CHO	F	250	4.11	3.23
4	CO_2Me[a]	F	264	4.24	3.07
5	COMe	F	267	4.23	3.17
6	NO_2	F	280	3.94	3.82
8	CO_2H	F	259	4.07	2.84
12	CH_2OH	F	250	4.39	
17	$CONH_2$	NO_2	250	4.53	3.84
19	CO_2Me[b]	NO_2	264	4.16	3.63
27	NO_2	NHMe	413	3.69	

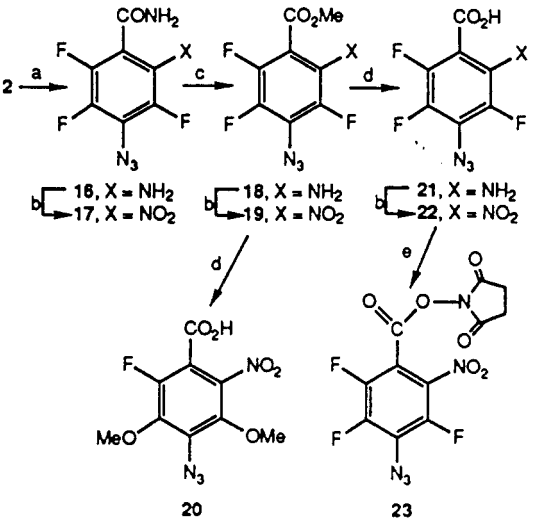
[a] λ (log ε): 240 (3.69), 280 (3.98), 320 (1.88), 340 (0.88). [b] λ (log ε): 240 (4.04), 280 (3.98), 320 (3.26), 340 (2.72).

has been used as a bifunctional cross-linking reagent in biochemistry,[33] it can be expected that **15a** should find similar application.

One disadvantage of these 4-substituted PFPA is that they all have UV absorption maxima around 260 nm (Table I) which is the region where biopolymers such as proteins and nucleic acids also have absorptions. Fortunately, because the photolabel reagent has a much greater molar absorptivity and photochemical lability than biopolymers, photolysis tends to be complete before any appreciable photolytic damage takes place. Since nitrophenyl azides such as 4-azido-*N*-methyl-2-nitroaniline have their absorption maxima at longer wavelengths,[34] it can be expected that the introduction of a nitro group meta to the azido should result in a bathochromic shift of the absorption maximum. Thus we sought to introduce a nitro group into a PFPA and to determine the effect of a nitro group[35] on the photochemical properties of PFPA.

Since pentafluoronitrobenzene[36] has been prepared by oxidation of pentafluoroaniline with peroxytrifluoroacetic acid (CF_3CO_3H),[37] we envisaged introduction of the nitro group via first introducing an amino group followed by oxidation. Pentafluoroaniline[38] has been prepared by reaction of $NaNH_2$ with hexafluorobenzene in liquid ammonia, and 2-aminotetrafluoronitrobenzene[36] has been prepared by treatment of pentafluoronitrobenzene with ammonia-saturated ether solution at 25 °C. However, we were unable to obtain any useful products from the reaction of amide **2** with $NaNH_2$ in liquid ammonia possibly because the azido group was attacked by $^-NH_2$. No reaction was observed when amide **2** was treated with ammonia-saturated ether solution at 25 °C for several days. Reasoning that the transition state might have more charge separation than the starting material, an ammonia-saturated dimethyl sulfoxide (DMSO) solution of amide **2** was kept at 75 °C for 5 days. Amine **16** was isolated as a pale orange solid in 49% yield. Oxidation of amine **16** with CF_3CO_3H gave nitro amide **17** (Scheme I) as a pale yellow

Scheme I[a]



[a] (a) NH_3/DMSO; (b) CF_3CO_3H; (c) Amberlyst 15/MeOH; (d) (1), NaOH/MeOH/H_2O, (2) HCl; (e) NHS/DCC.

solid in 63% yield. The UV spectrum of nitro azide **17** shows a maximum at 250 nm (log ε = 4.53, EtOH) with a shoulder absorption at 300 nm (log ε = 3.84) (Table I).

Amide **17** was designed to be a precursor of a series of activated carboxyl derivatives. However, hydrolysis of amide **17** under the conditions (Na_2O_2/H_2O, 50 °C)[23] used for the hydrolysis of amide **2** did not give any of the desired carboxylic acid, possibly because the nitro group rendered the ring sensitive to nucleophilic attack. Therefore amide **16** was converted into methyl ester **18** by refluxing in methanol in the presence of Amberlyst 15 resin.[39] Interestingly, amide **17** proved to be resistant to the esterification conditions. Oxidation of ester **18** with CF_3CO_3H gave nitro ester **19** as a pale yellow solid in 98% yield. Ester **19** shows a UV absorption maximum at 264 nm (log ε = 4.16) and a shoulder absorption at 300 nm (log ε = 3.63) (Table I).

The position of the amino substituent in amino amide **16** was assigned ortho to the amide based on ^{19}F NMR and IR spectra. The ^{19}F NMR spectrum of **16** shows that only one of the fluorine atoms has a complex pattern because of its coupling with the NH_2 of the amide, and this complex pattern is simplified by addition of D_2O. This assignment was supported by ^{19}F NMR spectra of **17** and **18** because nitro amide **17** shows the same complex pattern as **16** while amino ester **18** shows no such complex pattern. The assignment is also supported by IR spectra which show a shift of the carbonyl absorption from 1700 cm^{-1} for **2** to 1660 cm^{-1} for **16** because of intramolecular hydrogen bonding between the carbonyl and the ortho amino group. The ^{19}F NMR spectral data for the trisubstituted compounds are collected in the supplementary material.

Mild basic hydrolysis of ester **19** (see hydrolysis of ester **4**) gave acid **20** instead of the desired acid **22**. The position of the fluorine atom in **20** was assigned based on the assumption that the nitro group activated the ortho and para fluorines toward nucleophilic attack by methoxide ion. Acidic hydrolysis conditions (4 N HCl, up to 80 °C) applied to ester **19** led only to recovering of **19**, possibly because the initial protonation of the carbonyl group was rendered difficult by the electron-withdrawing substituents.

Fortunately, ester **18** underwent smooth hydrolysis under basic conditions to give amino acid **21** in 98% yield.

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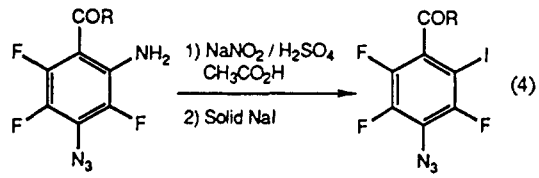
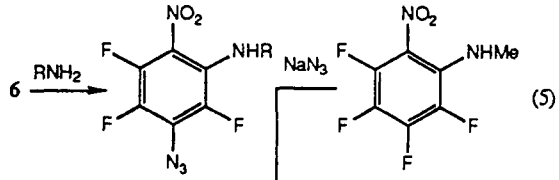
This amine was oxidized with CF_3CO_3H to give the desired nitro acid **22** as a solid in 72% yield. The *N*-hydroxysuccinimidyl ester **23** was prepared from acid **22** with NHS and DCC. Ester **23** should find application as a bifunctional cross-linking reagent similar to ester **11**.[27]

The amino group in ester **18** also was used to incorporate an iodine atom into the PFPA photolabel **24** via a diazotization reaction. A NHS ester **26** of this iodinated PFPA also was prepared (eq 4) for possible attachment to other molecules as described elsewhere.[17]



18, R = OMe
25, R = NHS
24, R = OMe
26, R = NHS

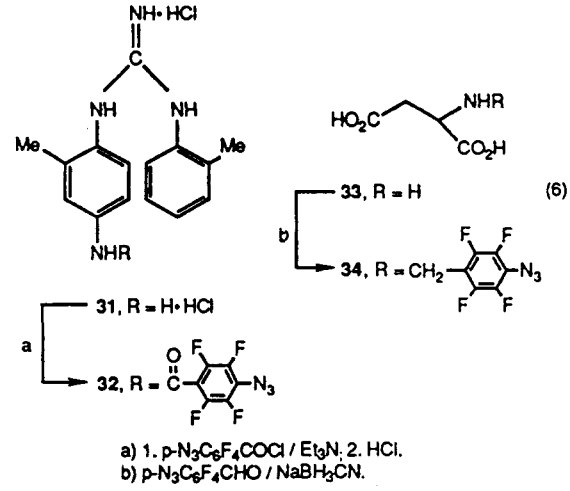
The popular reagent 4-fluoro-3-nitrophenyl azide[34] has been used to introduce a nitroaryl azide moiety into other molecules via nucleophilic replacement of the fluorine by an available amino group. Since pentafluoronitrobenzene is known to undergo nucleophilic substitution with amines,[40] we expected that 4-azidotetrafluoronitrobenzene[15] (**6**) should undergo the same reaction. In the event, azide **6** reacted with methylamine in ethanol/ether at 25 °C to give azide **27** as a yellow solid with λ_{max} = 413.5 nm (log ϵ = 3.69) in 74% yield. The position of the amino substituent in **27** was established via an independent synthesis of **27** by the reaction of *N*-methyl-2-aminotetrafluoronitrobenzene[41] (**30**) with sodium azide. Similarly prepared were azides **28** and **29** (eq 5). These reactions suggested that azide **6** could be used to introduce a PFPA moiety into other molecules through nucleophilic displacement of one of the fluorine atoms by an available amino group.



27, R = Me
28, R = i-Bu
29, R = p-Tol
30

The mammalian brain σ-receptor ligand *N,N'*-di-*o*-tolylguanidine (DTG)[42] was modified by a PFPA via the selective acylation of an amino derivative **31**[43] with acyl chloride **9** to give the amide derivative **32** (eq 6). Ligand **32** shows an IC_{50} = 25 nm against [^{3}H]DTG,[44] a value which is comparable to that of DTG itself and suggests that **32** may be a useful companion to photolabels already developed for the σ-receptor.[43]

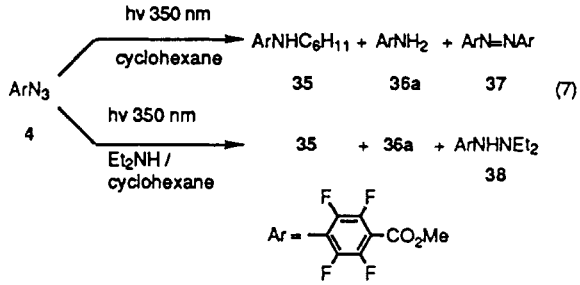
A photoactive analogue **34** of *N*-methyl-D-aspartic acid (NMDA)[45] was synthesized by reductive amination of D-aspartic acid (**33**)[46] with aldehyde **3** and $NaBH_3CN$.[47]



a) 1. p-$N_3C_6F_4COCl$ / Et_3N; 2. HCl.
b) p-$N_3C_6F_4CHO$ / $NaBH_3CN$.

This reaction demonstrates that the aldehyde group in azide **3** can be used for reductive amination without concomitant reduction of the azido group.

B. Photochemical Behavior of Functionalized Perfluorophenyl Azides. The photolytic behavior of two representative PFPA molecules, namely, azides **4** and **19**, was investigated, with the methyl group serving as a useful ^{1}H NMR marker. A solution of azide **4** in cyclohexane (5×10^{-3} M) was photolyzed (350 nm) to give amine **35** (57%), aniline **36a** (21%), and azobenzene **37** (11%) as the main products. The moderate yield of CH insertion product amine **35** shows that this functionalized PFPA largely retains the favorable photochemical behavior of pentafluorophenyl azide and is much better at undergoing CH insertion than nonfluorinated phenyl azides.[4,48] When the triplet sensitizer acetophenone (5×10^{-2} M) was present in the photolysis solution, the yield of amine **35** decreased to 14% while aniline **36a** increased to 66%, suggesting that the singlet nitrene is more efficient in undergoing CH insertion than the triplet nitrene. In the presence of diethylamine (5×10^{-2} M) as a trapping reagent,[5,6] the main photolytic products were substituted hydrazine **38** (65%), aniline **36a** (24%), and amine **35** (8%) (eq 7). Hydrazine **38** was identified by its AA'XX' pattern



in the ^{19}F NMR spectrum which excluded the possibility of ring expansion. These three products together accounted for greater than 95% of the starting material, and no trace of ring expansion product was observed. Therefore Dunkin's observation that the photochemically generated perfluorophenyl nitrene does not undergo ring expansion at low temperature could be extended to ambient temperature in the case of azide **4**.

Photolysis of azides **4** and **19** in toluene at room temperature was carried out to determine the effect of the nitro group on the photochemical behavior of PFPA. The

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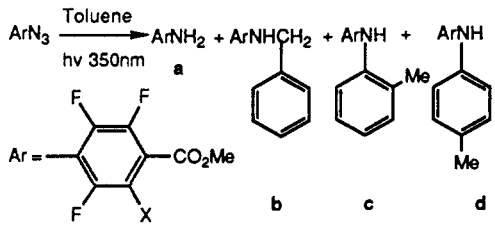
(48) Photolysis of phenyl azide in cyclohexane gave no CH insertion product. Reiser, A.; Leyshon, L. *J. Am. Chem. Soc.* **1971**, *93*, 4051.

Table II. The Products from Photolysis of Azides 4 and 19 in Toluene[a,b]



ArN_3	prod	X	time, h	yield, % a	b	c	d
4	36	F	5	29	16	13	9
4[c]	36	F	5	62	8	N[d]	N
19	39	NO_2	1.5	71	8	N	N

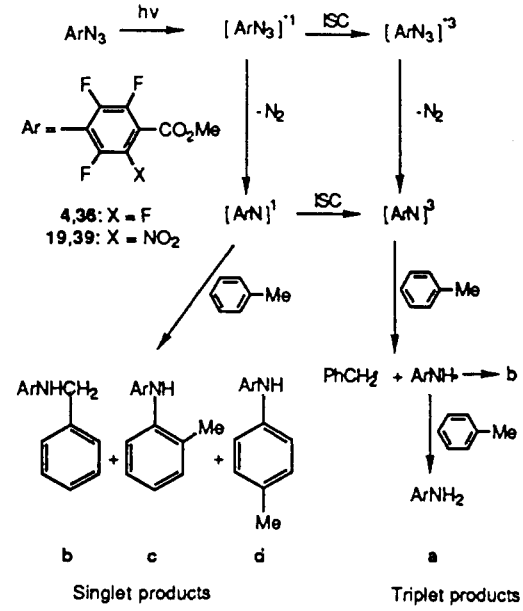
[a] λ = 350 nm, Rayonet photochemical reactor, 4.0×10^{-3} M. [b] The structures of amines **36b–36d** were established by reaction of ester **7** with the corresponding amines. [c] In the presence of 5.0×10^{-2} M acetophenone. [d] Not detected.

results are collected in Table II. It can be seen that replacement of one of the fluorine atoms by a nitro group brings several interesting changes. The photolysis time was decreased, likely the direct result of increased absorption at longer wavelengths. The yield of aniline **39a** increased, and no products of formal CH insertion into the ring[49] of toluene were observed. Photolysis of azide **4** in the presence of the triplet sensitizer acetophenone resulted in an increased yield of aniline **36a** and disappearance of amines **36c** and **36d**. The photolytic behavior of **4** in the presence of the triplet sensitizer was similar to that of **19** without the triplet sensitizer, suggesting that the presence of a nitro group promotes the formation of triplet products.[35] Nevertheless, the isolation of amine **39b** shows that nitro PFPA is much better at undergoing CH insertion than nonfluorinated nitro azides.[50]

Direct photolysis of phenyl azides can lead to singlet nitrenes and triplet nitrenes[6] (Scheme II). Since ring expansion was not observed for photochemically generated perfluorophenyl nitrenes, the singlet nitrenes can either undergo CH insertion to form secondary amines or intersystem crossing (ISC) to triplet nitrenes. The triplet nitrenes can abstract a hydrogen atom to form radical pairs which could then lead to either anilines or secondary amines, the former by successive hydrogen abstraction and the latter by radical recombination.[51] The increased yield of aniline **39a** compared to **36a** suggests that the nitro group might accelerate ISC of the singlet nitrene or the singlet excited azide[6] and so promote the formation of triplet products,[35] similar to the heavy atom effect.[52] That electron-withdrawing substituents could enhance the reactivity of triplet nitrenes toward hydrogen abstraction[53] may also promote the formation of triplet products.

The absence of amines c and d in the photolysis products derived from **19** constitutes additional evidence that the nitro group might accelerate ISC of the singlet nitrene or the singlet excited azide. Since the first step in CH insertion by triplet nitrenes is hydrogen abstraction, they should show a strong preference for abstraction of a benzylic hydrogen atom over a ring hydrogen atom in toluene because the ring CH bond is much stronger than the methyl CH bond.[54] Therefore triplet derived CH insertion products should have more amine b, less amines c and d. On the other hand, since CH insertion by singlet nitrenes is considered to be one step reaction[55] involving no hydrogen abstraction, singlet nitrenes should show less differentiation toward the methyl CH and the ring CH of toluene than that of triplet nitrenes and so should give comparable amounts of amine b, amines c and d.

Scheme II



It should be pointed out that the above discussion refers to solution chemistry in which the radicals are free to diffuse away and to abstract another hydrogen from other molecules instead of undergoing radical recombination. If the nitrenes are in an environment where motion is restricted, such as in the solid state,[10] or are part of a ligand bound to a macromolecular receptor, radical coupling could become dominant. Therefore nitro azide **19** might be a useful photolabel reagent despite its high yield of aniline product when photolyzed in solution.

In conclusion, the synthesis of functionalized PFPA **2–6**, **8–12**, **14–19**, and **21–29** provides several convenient alternatives for the attachment of PFPA to other bioactive molecules for application as photolabel reagents. Our photolysis results demonstrate that substituted PFPA give higher yields of CH insertion than their nonfluorinated analogues, possibly due to the higher reactivity of the nitrenes and the lack of ring expansion. With these desirable properties, functionalized PFPA should find application as a new series of photolabeling reagents. The application of these reagents for PAL is currently underway in this laboratory.

Experimental Section

^1H NMR spectra were measured at 300 MHz in $CDCl_3$ unless otherwise specified. ^{19}F NMR spectra (C_6F_6, −162.9 ppm as internal standard) were measured on a NT-360 spectrometer. IR spectra were recorded in $CDCl_3$. UV spectra were measured in

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ethanol on a Beckman DU-7 spectrometer. Melting points are uncorrected. All reactions involving azides were run under subdued light by wrapping the flasks with aluminum foil. Reagent grade solvents were used without further purification unless otherwise specified. All the C_6F_5X (X = CN, $CONH_2$, CHO, CO_2H, COMe, and NO_2) compounds were from Aldrich Co. Photolysis were carried out in a Rayonet photochemical reactor with 350-nm lamps at ambient temperature. Solutions were purged with Ar for 1 min before photolysis.

4-Azidotetrafluorobenzamide (2). (a) A mixture of 0.980 g (15.0 mmol) of NaN_3 and 3.04 g (14.4 mmol) of pentafluorobenzamide in acetone (20 mL) and water (7 mL) was refluxed for 8 h. The mixture was cooled and then added to water (20 mL). The white precipitate was filtered, washed by water, and then dried at 25 °C by aspirator to give 2.81 g (83%) of **2** as white powder. ^1H NMR: δ 5.90 (broad). IR: 3525, 3405, 2135, 1700, 1486, 996 cm^{-1}. Crystallization (CH_3CN/hexane) gave the analytical sample of **2** as white needles, mp 164–165 °C dec. Anal. Calcd for $C_7H_2F_4N_4O$: C, 35.91; H, 0.86; N, 23.93. Found: C, 35.96; H, 0.68; N, 24.16.

(b) To a stirred solution of 7.6 g (35 mmol) of **1**[13] in CH_2Cl_2 (30 mL) cooled in an ice bath was added 30% H_2O_2 (20 mL), followed by 2.6 g (7.5 mmol) of $(n\text{-}C_4H_9)_4NHSO_4$ and 20% aqueous NaOH (17 mL, 3.4 g, 85 mmol). The mixture was then stirred at 25 °C for 40 h. The precipitate was filtered, washed by CH_2Cl_2 (2 mL), and then dried by aspirator to leave 1.54 g (18%) of white powder, mp 164–165 °C. The ^1H NMR and IR spectra are identical with those for **2** obtained by method a.

4-Azidotetrafluorobenzaldehyde (3). A mixture of 0.30 g (4.6 mmol) of NaN_3 and 0.88 g (4.3 mmol) of pentafluorobenzaldehyde in acetone (8 mL) and water (3 mL) was refluxed for 8 h. The mixture was cooled, diluted with water (10 mL), and then extracted by ether (3 × 10 mL). The extract was dried ($MgSO_4$) and evaporated to leave 0.93 g (95%) of pale red liquid which solidified at standing, mp 44–45 °C. ^1H NMR: δ 10.233 (m). IR: 2161, 2123, 1710, 1644, 1494, 1486, 1401, 1241, 992 cm^{-1}. Sublimation (25 °C/0.1 mm) gave the analytical sample of **3** as a colorless solid, mp 44–45 °C. Anal. Calcd for $C_7HF_4N_3O$: C, 38.37; H, 0.46; N, 19.18. Found: C, 38.14; H, 0.26; N, 19.56.

The following azides (**4–6**) were prepared from the corresponding pentafluoro precursor as described above for **3**.

Methyl 4-Azidotetrafluorobenzoate (4). Colorless solid (87%), mp 54–55 °C. ^1H NMR: δ 3.966 (s). IR: 2958, 2127, 1738, 1491, 1261, 911 cm^{-1}. Anal. Calcd for $C_8H_3F_4N_3O_2$: C, 38.57; H, 1.21; N, 16.87. Found: C, 38.53; H, 1.09; N, 16.91.

4′-Azido-2′,3′,5′,6′-tetrafluoroacetophenone (5). Colorless liquid (80%), bp 50.0–52.0 °C/0.1 mm. ^1H NMR: δ 2.608 (t, J_{FH} = 1.77). IR: 2132, 1707, 1643, 1485, 1247 cm^{-1}. Anal. Calcd for $C_8H_3F_4N_3O$: C, 41.22; H, 1.30; N, 18.02. Found: C, 41.08; H, 1.21; N, 17.91.

4-Azidotetrafluoronitrobenzene (6).[15] Pale yellow liquid (71%), bp 59–61 °C/0.01 mm. IR: 2159, 2126, 1639, 1555, 1514, 1499, 1486, 1356, 1308, 1233, 1013 cm^{-1}. ^{19}F NMR revealed the presence of <5% of ortho isomer. ^{19}F NMR of the ortho isomer: δ −147.21, −149.74, −150.31, −157.74.

Methyl Pentafluorobenzoate (7).[22] To a solution of 4.56 g of pentafluorobenzoic acid and MeOH (5 mL) in CCl_4 (35 mL) was added concentrated H_2SO_4 (1.5 mL). The mixture was refluxed overnight. The cooled mixture was washed by 5% Na_2CO_3 (3 × 13 mL) and water (2 × 10 mL), dried ($MgSO_4$), and evaporated to leave 4.69 (96%) of **7** as a colorless liquid. ^1H NMR: δ 3.982 (s). IR: 2958, 1742, 1524, 1501, 1333, 1241, 1010, 979 cm^{-1}.

4-Azidotetrafluorobenzoic Acid (8). (a) A mixture of 0.56 g of **2** (2.4 mmol) and 0.24 g (3.0 mmol) of Na_2O_2 with water (10 mL) was heated at 50 °C for 42 h. The mixture was cooled in an ice bath and slowly acidified by concentrated HCl to pH <1. The precipitate was filtered and dried by aspirator at 25 °C to yield 0.54 g of almost colorless solid, which was purified by chromatography (silica gel, 5:5:2 pentane–CH_2Cl_2–CH_3CN) to give 0.37 g (64%) of **8** as colorless solid, mp 140–141 °C. IR: 3000 (b), 2136, 1747, 1715, 1646, 1491, 1423, 1263, 1004 cm^{-1}. Anal. Calcd for $C_7HF_4N_3O_2$: C, 35.76; H, 0.43; N, 17.87. Found: C, 35.69; H, 0.22; N, 17.84.

(b) A solution of 0.586 g of **4** with 20% aqueous NaOH (0.8 mL) in MeOH (10 mL) and water (1 mL) was stirred overnight at 25 °C. The solution was acidified by 2 N HCl in an ice bath to pH <1 and extracted by $CHCl_3$ (3 × 10 mL). The extract was dried ($MgSO_4$) and evaporated to leave 0.54 g (98%) of **8** as a colorless solid, mp 140–141 °C. The IR spectrum is identical with that for **8** obtained by method a.

4-Azidotetrafluorobenzoyl Chloride (9). A solution of 2.97 g of **8** and $SOCl_2$ (2 mL) in dry CH_2Cl_2 (30 mL) was refluxed for 36 h. The solvent was removed by aspirator at 25 °C, and the residue was distilled to give 2.25 g (70%) of **9** as a yellow liquid, bp 46.5–49.0 °C/0.01 mm. IR: 2125, 1765, 1644, 1493, 1241, 1006 cm^{-1}. Anal. Calcd for $C_7ClF_4N_3O$: C, 33.16; H, 0.00; N, 16.57. Found: C, 33.30; H, 0.09; N, 16.86.

***N*-[(4-Azidotetrafluorophenyl)carbonyl]imidazole (10).** A solution of 241 mg (1.02 mmol) of **8** and 167 mg (1.03 mmol) of 1,1′-carbonyldiimidazole in dry THF (4 mL) was stirred for 1.5 h, and the solvent was removed by aspirator at 25 °C to leave a pale yellow liquid. The liquid was filtered through a short silica gel column (1:1 CH_2Cl_2–ether). The filtrate was evaporated to leave 206 mg (70%) of **10** as a pale yellow oil, which crystallized at standing, mp 63–64 °C. ^1H NMR: δ 7.189 (s, 1), 7.458 (s, 1), 7.975 (s, 1). IR: 2130, 1705, 1692, 1618, 1500 cm^{-1}. MS (rel intensity): 285 (10, M^+), 218 (55), 162 (100). High-resolution MS calcd for $C_{10}H_3F_4N_5O$ 285.0274, found 285.0267.

***N*-Succinimidyl 4-Azidotetrafluorobenzoate (11).** A solution of 234 mg (1.00 mmol) of **8**, 115 mg (1.00 mmol) of *N*-hydroxysuccinimide (NHS), and 211 mg (1.02 mmol) of dicyclohexylcarbodiimide (DCC) in CH_2Cl_2 (6.5 mL, redist) was stirred at 25 °C overnight. The mixture was filtered. The filtrate was evaporated to leave 331 mg (99%) of **11** as a colorless solid, mp 103–104 °C. ^1H NMR: δ 2.919 (s). IR: 2128, 1784, 1749, 1649, 1494, 1240, 1202, 1129, 1012 cm^{-1}. Crystallization ($CHCl_3$/hexane) gave the analytical sample of **11** as colorless needles, mp 105–106 °C. Anal. Calcd for $C_{11}H_4F_4N_4O_4$: C, 39.79; H, 1.21; N, 16.87. Found: C, 40.24; H, 1.21; N, 16.72.

4-Azidotetrafluorobenzyl Alcohol (12). A stirred solution of 1.05 g (4.79 mmol) of **3** and 0.340 g (5.76 mmol) of $Me_2NH{\cdot}BH_3$ in acetic acid (15 mL) was heated at 55 °C for 1 h. The solvent was removed by aspirator at 45 °C. The residue was dissolved in $CHCl_3$ (20 mL), washed by 5% Na_2CO_3 (2 × 15 mL) and water (15 mL), dried ($MgSO_4$), and evaporated to leave 1.04 g (98%) of colorless solid, mp 67–68 °C. ^1H NMR: δ 1.922 (sb, 1), 4.790 (s, 2). IR: 3644, 2150, 2121, 1653, 1497, 1238, 1007 cm^{-1}. Sublimation (50 °C/0.05 mm) gave the analytical sample of **12** as a colorless solid, mp 67–68 °C. Anal. Calcd for $C_7H_3F_4N_3O$: C, 38.02; H, 1.37; N, 19.00. Found: C, 37.93; H, 1.30; N, 18.60.

4-Azidotetrafluorobenzyl Acetate (13). To a solution of 109 mg (0.49 mmol) of **12** and 50 mg (0.50 mmol) of Et_3N in ether (3 mL) was added a solution of 51 mg (0.65 mmol) of acetyl chloride in ether (1 mL). The mixture was stirred for 1 h, diluted by ether (10 mL), washed by water (3 × 7 mL), dried ($MgSO_4$), and evaporated to leave a liquid. It was purified by preparative TLC (1:2 acetone–hexane) to give 101 mg (78%) of **13** as a colorless liquid, which solidified in the freezer, mp 33–34 °C. ^1H NMR: δ 2.082 (s, 3), 5.171 (s, 2). IR: 2123, 1746, 1652, 1496, 1240 cm^{-1}. Anal. Calcd for $C_9H_5F_4N_3O_2$: C, 41.08; H, 1.92; N, 15.97. Found: C, 41.01; H, 1.68; N, 15.68.

4-Azidotetrafluorobenzylamine (14). To 865 mg (4.0 mmol) of nitrile **1**[13] in an ice bath was added 1 M THF·BH_3 solution (4.1 mL, 4.1 mmol) dropwise by syringe. The solution was stirred in the ice bath for 2 h, and then 2 N HCl (6 mL) was added dropwise. The mixture was extracted by ether (3 × 3 mL). The aqueous was basified by 2 N KOH to pH >12 and extracted by $CHCl_3$ (3 × 15 mL). The $CHCl_3$ extract was dried ($MgSO_4$) and evaporated to leave 601 mg of liquid, which was separated by preparative TLC (1:4:2 hexane–THF–$CHCl_3$) to leave 424 mg (48%) of **14** as a colorless liquid. ^1H NMR: δ 3.947 (s). IR: 2123, 1651, 1494, 1237, 994 cm^{-1}. MS (rel intensity): 220 (12, M^+), 204 (4), 192 (100). High-resolution MS calcd for $C_7H_4F_4N_4$ 220.0372, found 220.0370.

1-(4-Azidotetrafluorophenyl)-2-bromoethanone (15a) and 1-(4-Azidotetrafluorophenyl)-2,2-dibromoethanone (15b). A solution of 1.02 g (6.40 mmol) of Br_2 in ether (2 mL) was added dropwise to a solution of 1.47 g (6.30 mmol) of **5** in ether (10 mL) in an ice bath. The solution was stirred at 25 °C for 4 h, washed by water (5 × 15 mL), dried ($MgSO_4$), and evaporated to leave 1.92 g of liquid. ^1H NMR spectroscopy showed that it was a mixture of **5**, **15a**, and **15b** in a ratio of 0.05:0.90:0.05. A portion of the mixture was separated by preparative TLC (2:3

CH_2Cl_2–hexane) to leave **15a** (R_f = 0.65) as a colorless liquid (71%). ^1H NMR: δ 4.309 (s). IR: 2133, 1707, 1645, 1488, 1416, 1251, 1092, 1001 cm^{-1}. Anal. Calcd for $C_8H_2BrF_4N_3O$: C, 30.79; H, 0.65; N, 13.47. Found: C, 30.86; H, 0.47; N, 13.42. **15b** (R_f = 0.80) was isolated as a colorless solid, mp 42–43 °C. ^1H NMR: δ 6.535 (s). IR: 2134, 1723, 1645, 1490, 1411, 1324, 1251, 1231, 1002 cm^{-1}. MS (rel intensity): 393, 391, 389 (1, 2, 1, M^+), 365, 363, 361 (1, 2, 1), 337, 335, 333 (2, 4, 2), 284, 282 (2, 2), 256, 254 (7, 7), 218 (100), 175 (40), 162 (70). High-resolution MS calcd for $C_8HBr_2F_4N_3O$ 390.8402, found 390.8400.

2-Amino-4-azido-3,5,6-trifluorobenzamide (16). Through a stirred solution of 10.24 g of **2** in dry DMSO (70 mL) at 75 °C was bubbled NH_3 slowly for 5 days. The solution was cooled and then added dropwise to water (350 mL), and the mixture was stirred for 1 h. The precipitate was filtered and washed by water (50 mL) and then dried by aspirator at 45 °C to leave 4.92 g (49%) of pale orange solid, mp 160–161 °C. ^1H NMR: δ 5.69 (m, 1), 6.05 (m, 2), 6.48 (m, 1). IR: 3545, 3502, 3421, 3348, 2129, 1667, 1648, 1493, 1465 cm^{-1}. Crystallization (acetone/hexane) gave the analytical sample of **16** as pale orange needles, mp 160–161 °C. Anal. Calcd for $C_7H_4F_3N_5O$: C, 36.37; H, 1.74; N, 30.29. Found: C, 36.54; H, 1.63; N, 30.15.

4-Azido-2-nitro-3,5,6-trifluorobenzamide (17). The CF_3CO_3H solution was prepared from 90% H_2O_2 (1.5 mL) (*caution!*) and trifluoroacetic anhydride (2.5 mL) in CH_3CN (2 mL) according to the known procedure.[37] To a stirred suspension of 500 mg of **16** in CH_3CN (8 mL) was added dropwise the CF_3CO_3H solution. The solution was heated at 55 °C for 6 h and then diluted by water (5 mL). This was extracted by a mixed solvent (1:1 methyl ethyl ketone–$CHCl_3$, 3 × 15 mL). The extract was washed by water (3 × 20 mL), dried ($MgSO_4$), evaporated, and put under vacuum (0.05 mm) overnight to leave 356 mg (63%) of **17** as a solid. ^1H NMR: δ 6.121 (m). IR: 3520, 3407, 2137, 1706, 1633, 1591, 1559, 1491, 1473, 1395 cm^{-1}. Crystallization (acetone/pentane) gave the analytical sample of **17** as a pale yellow solid, mp 135–136 °C. Anal. Calcd for $C_7H_2F_3N_5O_3$: C, 32.19; H, 0.77; N, 26.82. Found: C, 32.38; H, 0.69; N, 26.62.

Methyl 2-Amino-4-azido-3,5,6-trifluorobenzoate (18). A mixture of 0.664 g of **16** and 15 g of Amberlyst 15 resin with methanol (20 mL) was refluxed for 70 h. The mixture was cooled, filtered, and washed by ether (40 mL). The filtrate was evaporated to leave 0.585 g (88%) of solid, mp 74–75 °C. Sublimation (50 °C/0.05 mm) gave the analytical sample of **18** as a pale yellow solid, mp 82–83 °C. ^1H NMR: δ 3.919 (s, 3), 5.716 (m, 2). IR: 3511, 3387, 2128, 1694, 1646, 1592, 1492, 1477, 1294, 1270 cm^{-1}. Anal. Calcd for $C_8H_5F_3N_4O_2$: C, 39.03; H, 2.04; N, 22.76. Found: C, 39.13; H, 1.85; N, 22.64.

Methyl 4-Azido-2-nitro-3,5,6-trifluorobenzoate (19). The CF_3CO_3H solution was prepared in the same way as in **17**. To a stirred solution of 182 mg of **18** in CH_2Cl_2 (10 mL) was added dropwise the CF_3CO_3H solution. The mixture was refluxed for 6 h, cooled, washed by water (3 × 20 mL), dried ($MgSO_4$), and evaporated to leave 200 mg of yellow liquid (98%) which crystallized at standing, mp 66–67 °C. Sublimation (55 °C/0.03 mm) gave the analytical sample of **19** as a pale yellow solid, mp 67–68 °C. ^1H NMR: δ 3.964 (s). IR: 2958, 2130, 1749, 1557, 1479, 1385, 1256 cm^{-1}. Anal. Calcd for $C_8H_3F_3N_4O_4$: C, 34.79; H, 1.09; N, 20.28. Found: C, 35.06; H, 0.94; N, 20.25.

4-Azido-6-fluoro-3,5-dimethoxy-2-nitrobenzoic Acid (20). Ester **19** was hydrolyzed in a manner similar to ester **4** to give **20** as a solid (99%), mp 115–116 °C. ^1H NMR: δ 3.93 (s, 3), 4.09 (d, J = 1.8, 3). ^{19}F NMR: δ −130.65. IR: 3000 (b), 2126, 1744, 1716, 1552, 1486, 1379 cm^{-1}. Anal. Calcd for $C_9H_7FN_4O_6$: C, 37.77; H, 2.46; N, 19.57. Found: C, 37.78; H, 2.37; N, 19.17.

2-Amino-4-azido-3,5,6-trifluorobenzoic Acid (21). Ester **18** was hydrolyzed in a manner similar to ester **4** to give acid **21** as a colorless solid (98%), mp 170–171 °C. ^1H NMR: δ 5.844 (sb). IR: 3511, 3386, 2126, 1705, 1646, 1478, 1267 cm^{-1}. Anal. Calcd for $C_7H_3F_3N_4O_2$: C, 36.22; H, 1.30; N, 24.13. Found: C, 36.10; H, 1.17; N, 23.73.

4-Azido-2-nitro-3,5,6-trifluorobenzoic Acid (22). Acid **21** was oxidized by CF_3CO_3H in a manner similar to ester **18** to give acid **22** as a pale yellow solid (72%), mp 128–129 °C. IR: 3000, 2139, 1750, 1733, 1633, 1559, 1478, 1248 cm^{-1}. Anal. Calcd for $C_7HF_3N_4O_4$: C, 32.07; H, 0.38; N, 21.37. Found: C, 32.30; H, 0.26; N, 21.56.

***N*-Succinimidyl 4-Azido-2-nitro-3,5,6-trifluorobenzoate (23).** NHS ester **23** was prepared from acid **22** in a manner similar to ester **11** as a pale yellow solid (57%), mp 138–139 °C. ^1H NMR: δ 2.911 (s). IR: 2140, 1784, 1751, 1561, 1492, 1481, 1389, 1197 cm^{-1}. MS (rel intensity): 359 (1, M^+), 245 (92), 143 (100). High-resolution MS calcd for $C_{11}H_4F_3N_5O_6$ 359.0108, found 359.0087.

***N*-Methyl-2-amino-4-azido-3,5,6-trifluoro-1-nitrobenzene (27).** (a) A solution of 178 mg (0.756 mmol) of **6**, 60.1 mg (0.890 mmol) of $MeNH_2$·HCl, and Et_3N (0.5 mL) in EtOH (4 mL) and ether (2 mL) was stirred at 25 °C overnight, diluted by ether (10 mL), washed by water (3 × 10 mL), dried ($MgSO_4$), and evaporated to leave 195 mg of red powder. The solid was sublimated (55 °C/0.01 mm) to give 132.0 mg (71%) of **27** as a yellow solid, mp 68–69 °C. ^1H NMR: δ 3.138 (dd, J_{HF} = 6.61, J_{HH} = 5.56, 3), 6.6 (m, 1). IR: 3400, 2128, 1638, 1590, 1551, 1515, 1482, 1277, 1245 cm^{-1}. Anal. Calcd for $C_7H_4F_3N_5O_2$: C, 34.02; H, 1.63; N, 28.33. Found: C, 34.20; H, 1.48; N, 28.55.

(b) Azide **27** was prepared in a manner similar to azide **3** via reaction of **30**[41] with NaN_3 and isolated as a yellow solid, mp 68–69 °C. The ^1H NMR and IR spectra were identical with those for **27** obtained by method a.

***N*-(2-Methylpropyl)-2-amino-4-azido-3,5,6-trifluoro-1-nitrobenzene (28).** Reaction of azide **6** with isobutylamine was carried out similar to **6** with methylamine. The reaction mixture after workup was purified by preparative TLC (1:8 acetone–hexane) to give **28** as a yellow oil (60%). ^1H NMR: δ 0.964 (d, J = 6.6, 6), 1.834 (m, 1), 3.250 (m, 2), 6.663 (bs, 1). IR: 3383, 2984, 2933, 2876, 2129, 1637, 1587, 1552, 1510, 1470, 1286, 1274, 1239 cm^{-1}. Anal. Calcd for $C_{10}H_{10}F_3N_5O_2$: C, 41.52; H, 3.48; N, 24.21. Found: C, 41.14; H, 3.24; N, 23.90.

***N*-*p*-Tolyl-2-amino-4-azido-3,5,6-trifluoro-1-nitrobenzene (29).** A solution of 94 mg (0.40 mmol) of **6** and 83 mg (0.77 mmol) of *p*-toluidine in CH_3CN (3 mL) was refluxed for 18 h. The solution was diluted by ether (10 mL), washed by water (2 × 10 mL), dried ($MgSO_4$), and evaporated to leave a red oil which was purified by preparative TLC (1:1 CH_3Cl–hexane) to give 77 mg (60%) of **29** as a red solid, mp 74–75 °C. ^1H NMR: δ 2.322 (s, 3), 6.834 (dd, J = 2.0, 8.3, 2), 7.104 (d, J = 8.3, 2), 7.552 (m, 1). IR: 3386, 2129, 1546, 1516, 1484, 1299, 1009 cm^{-1}. Anal. Calcd for $C_{13}H_8F_3N_5O_2$: C, 48.31; H, 2.49; N, 21.67. Found: C, 48.15; H, 2.44; N, 21.71.

Acylation of *N*-(2-Methyl-4-aminophenyl)-*N'*-(2-methylphenyl)guanidine (Amino-DTG, 31) by 4-Azidotetrafluorobenzoyl Chloride (9). Synthesis of 32. A mixture of 215 mg (0.650 mmol) of amino-DTG·2HCl[43] and 74 mg (0.73 mmol) of dry Et_3N in dry DMF (10 mL) was stirred for 20 min under N_2. A solution of 173 mg (0.68 mmol) of **9** in DMF (1 mL) was added and stirred for 5 min, and then 66 mg (0.65 mmol) of Et_3N was added. It was stirred for 5 h, 70 mg (0.69 mmol) of Et_3N was added, and then the mixture was stirred for 1 h. The solution was diluted by $CHCl_3$ (20 mL), washed by water (4 × 10 mL), dried ($MgSO_4$), and evaporated to leave a yellow oil, which was further dried by vacuum (0.1 mm). The oil was purified by preparative TLC (2:0.5:0.01 CH_2Cl_2–MeOH–CH_3CO_2H) to give 170 mg of semisolid (55% crude yield). It was dissolved in 7 mL of MeOH and filtered, and then 0.3 mL of 6.8 N methanolic HCl solution was added. The solution was evaporated to leave a solid, which was further dried by vacuum (0.1 mm). The solid was crystallized (methanol/ether) to give **32** as a colorless solid (67 mg), mp 170–171 °C. ^1H NMR (CD_3OD): δ 2.36 (s, 3), 2.37 (s, 3), 7.30 (m, 5), 7.64 (dd, J = 2.4, 8.4, 1), 7.69 (d, J = 2.4, 1). Anal. Calcd for $C_{22}H_{17}F_4N_7O$·HCl: C, 52.02; H, 3.57; N, 19.30. Found: C, 51.63; H, 3.50; N, 19.07.

***N*-(4-Azidotetrafluorobenzyl)-D-aspartic Acid (34).** To a solution of 33 mg (0.52 mmol) of $NaBH_3CN$ and 493 mg (3.70 mmol) of D-aspartic acid in buffer (15 mL, pH = 7.5, 1 M NaH_2PO_4) was added a solution of 173 mg (0.79 mmol) of aldehyde **3** in methanol (4 mL). The solution was stirred at 25 °C overnight and extracted by $CHCl_3$ (3 × 10 mL). The aqueous layer was acidified by 6 N HCl to pH = 4 and extracted by ethyl acetate (3 × 15 mL). The ethyl acetate extract was evaporated to leave 82 mg of white solid (31% based on **3**). ^1H NMR (D_2O): δ 2.90 (dd, J = 7.5, 18.3, 1), 3.02 (dd, J = 4.2, 18.3, 1), 3.956 (dd, J = 4.2, 7.5, 1), 4.49 (m, 2). IR (Nujol): 3200, 2129, 1733, 1654, 1576, 1494, 1238 cm^{-1}. The analytical sample of **34** was obtained by

crystallization from water as a white solid, mp 170–171 °C dec. Anal. Calcd for $C_{11}H_8F_4N_4O_4$: C, 39.30; H, 2.40; N, 16.66. Found: C, 39.73; H, 2.10; N, 16.49.

Photolysis of Azide 4 in Cyclohexane. A 5.0×10^{-3} M solution of azide **4** in cyclohexane was photolyzed for 4.5 h. The crude photolysis mixture was separated by preparative TLC (3:2 $CHCl_3$–hexane) to give amine **35** (57%, R_f = 0.35), aniline **36a** (21%, R_f = 0.14), and azobenzene **37** (11%, R_f = 0.50). Amine **35**. ^{1}H NMR: δ 1.2 (m, 3), 1.3 (m, 2), 1.6 (m, 1), 1.76 (m, 2), 2.05 (m, 2), 3.6 (m, 1), 3.904 (s, 3), 4.02 (m, 1). Sublimation (60 °C/0.05 mm) gave the analytical sample of **35** as a colorless solid, mp 86–87 °C. Anal. Calcd for $C_{14}H_{15}F_4NO_2$: C, 55.08; H, 4.95; N, 4.59. Found: C, 55.28; H, 4.76; N, 4.48. Aniline **36a**: mp 113–114 °C (lit.[56] mp 116.5–117.0 °C). ^{1}H NMR: δ 3.916 (s, 3), 4.35 (s, 2). Azobenzene **37**. ^{1}H NMR: δ 4.032 (s). MS (rel intensity): 442 (44, M^+), 411 (23), 235 (70), 207 (100), 192 (30), 176 (50), 148 (52). High-resolution MS calcd for $C_{16}H_6F_8N_2O_4$ 442.0198, found 442.0200.

Photolysis of Azide 4 in Cyclohexane in the Presence of the Triplet Sensitizer Acetophenone. A 5.0×10^{-3} M solution of azide **4** in cyclohexane containing 5.0×10^{-2} M of acetophenone was photolyzed for 5 h. ^{1}H NMR of the crude photolysis mixture showed amine **35** (14%) and aniline **36a** (66%).

Photolysis of Azide 4 in Cyclohexane in the Presence of Diethylamine as Trapping Reagent. A 5.0×10^{-3} M solution of azide **4** in cyclohexane containing 5.0×10^{-2} M of diethylamine was photolyzed for 5 h. The crude photolysis mixture was separated by preparative TLC (2:1 $CHCl_3$–hexane) to give amine **35** (8%), aniline **36a** (24%), and hydrazine **38** (65%, R_f = 0.33). Hydrazine **38**. ^{1}H NMR: δ 1.105 (t, J = 6.9, 6), 2.753 (q, J = 6.9, 4), 3.916 (s, 3), 4.593 (s, 1). Sublimation (70 °C/0.05 mm) gave the analytical sample of **38** as a colorless solid, mp 94–95 °C. Anal. Calcd for $C_{12}H_{14}F_4N_2O_2$: C, 48.98; H, 4.80; N, 9.52. Found: C, 49.05; H, 4.63; N, 9.47.

Photolysis of Azide 4 in Toluene. A 4.0×10^{-3} M solution of azide **4** in toluene was photolyzed for 5 h. The crude photolysis mixture was separated by preparative TLC (2:1 $CHCl_3$–hexane) to give aniline **36a** (29%), amine **36b** (16%), **36c** (13%), and **36d** (9%). The structure of amine **36b–d** were established by reaction of the corresponding amine with ester **7** as described next.

Methyl *N*-Benzyl-4-aminotetrafluorobenzoate (36b). A solution of 130 mg of ester **7**, 90 mg of benzylamine, and 80 mg of Et_3N in 10 mL of CH_3CN was refluxed for 3 h. The solvent was evaporated, and the residue was dried by vacuum (0.05 mm, 25 °C). The solid was then sublimated (0.05 mm, 70 °C) to give 101 mg of **36b** as a white solid, mp 95–96 °C. ^{1}H NMR: δ 3.906 (s, 3), 4.478 (m, 1), 4.643 (d, J = 6.0, 2), 7.337 (m, 5). Anal. Calcd for $C_{15}H_{11}F_4NO_2$: C, 57.52; H, 3.54; N, 4.47. Found: C, 57.59; H, 3.53; N, 4.27.

Methyl *N*-(2-methylphenyl)-4-aminotetrafluorobenzoate (36c) and methyl *N*-(4-methylphenyl)-4-aminotetrafluorobenzoate (36d) were synthesized from ester **7** and the corresponding amines in a manner similar to **36b**. Amine **36c**: mp 110–111 °C; ^{1}H NMR: δ 2.328 (s, 3), 3.950 (s, 3), 5.620 (s, 1), 6.938 (d, J = 7.2, 1), 7.084 (t, J = 7.2, 1), 7.177 (t, J = 7.7, 1), 7.220 (d, J = 7.7, 1). Anal. Calcd for $C_{15}H_{11}F_4NO_2$: C, 57.52; H, 3.54; N, 4.47. Found: C, 57.96; H, 3.44; N, 4.58. Amine **36d**: mp 136–137 °C. ^{1}H NMR: δ 2.334 (s, 3), 3.948 (s, 3), 5.908 (s, 1), 6.914 (d, J = 8.0, 2), 7.127 (d, J = 8.0, 2). Anal. Calcd for $C_{15}H_{11}F_4NO_2$: C, 57.52; H, 3.54; N, 4.47. Found: C, 57.75; H, 3.31; N, 4.30.

Photolysis of Azide 4 in Toluene in the Presence of the Triplet Sensitizer Acetophenone. A 4.0×10^{-3} M solution of azide **4** in toluene containing 5.0×10^{-2} M of acetophenone was photolyzed for 5 h. ^{1}H NMR of the crude photolysis mixture showed aniline **36a** (62%) and amine **36b** (8%). No **36c** and **36d** were observed.

Photolysis of Azide 19 in Toluene. Photolysis of azide **19** and separation of the reaction mixture were carried out in a manner similar to azide **4** except that the photolysis time was 1.5 h. Aniline **39a** (71%, R_f = 0.21), mp 116–117 °C. ^{1}H NMR: δ 3.893 (s, 3), 4.52 (m, 2). Anal. Calcd for $C_8H_5F_3N_2O_4$: C, 38.41; H, 2.01; N, 11.20. Found: C, 38.29; H, 1.90; N, 10.86. Amine **39b** (8%, R_f = 0.27). ^{1}H NMR: δ 3.878 (s, 3), 4.660 (s, 2), 5.61 (sb, 1), 7.33 (m, 5). MS (rel intensity): 340 (20, M^+), 309 (5), 91 (100). High-resolution MS calcd for $C_{15}H_{11}F_3N_2O_4$ 340.0668, found 340.0670.

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Supplementary Material Available: Tables of the ^{19}F NMR spectral data for 1,4-disubstituted compounds **2–5**, **8–15b**, and **35–38** and trisubstituted compounds **16–19**, **21–23**, **27–29**, and **39a–b** and ^{1}H NMR (300 MHz) spectra of compounds **10**, **14**, **15b**, **23**, **37**, and **39b** (8 pages). Ordering information is given on any current masthead page.

Metal–1,10-Phenanthroline-Linked Dihydronicotinamides as Models for the NADH–Alcohol Dehydrogenase Coenzyme–Enzyme Couple

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Two phenanthroline-linked dihydronicotinamides, 1,4-dihydro-1-(1,10-phenanthrolin-2-ylmethyl)-3-pyridinecarboxamide (**3**) and 1,4-dihydro-*N*-(1,10-phenanthrolin-2-ylmethyl)-1-(phenylmethyl)-3-pyridinecarboxamide (**6**), were synthesized. The phenanthroline moiety in these compounds is able to chelate metal ions into fixed positions toward the dihydronicotinamide group providing models for the NADH–alcohol dehydrogenase complex. The reactivity of **3**–M^{2+} and **6**–M^{2+} is investigated toward 2,4,6-trinitrobenzene sulfonate and methylene blue (M^{2+} = Zn^{2+}) and toward the metallophilic substrate 2-pyridinecarboxaldehyde (PyCHO) for which a detailed kinetic analysis is given. For reaction of **3**–M^{2+} and PyCHO the efficiency of metal ion activation is of the order $Zn^{2+} \gg Mg^{2+}$, $Ni^{2+} > Co^{2+} > Cd^{2+}$. It is concluded that hydride transfer proceeds in a ternary **3**–M^{2+}–PyCHO complex in an orientation in which carbonyl group and dihydropyridine are in a coplanar position with the carbonyl oxygen pointing to the ring nitrogen.

The enzyme alcohol dehydrogenase is one of the dehydrogenases that utilizes zinc ion at the active site as a catalytic group. It catalyses the transfer of a hydride equivalent from the coenzyme NADH to a large variety of aliphatic and aromatic aldehydes and ketones and reverse. Alcohol dehydrogenase from horse liver, human liver, rat liver, yeast, and bacillus have been extensively studied and characterized, both by X-ray and chemical

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