Synthesis of Photoaffinity Probes for Heme-Containing Proteins

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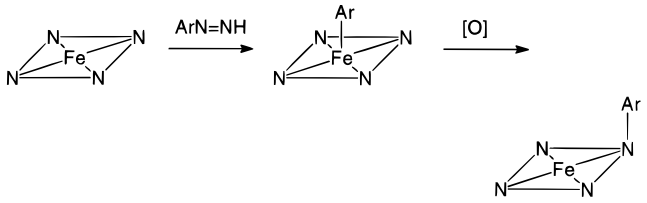
Aryldiazenes with a second, photoactivatable, azide substituent on the aryl ring have been synthesized as active site probes for heme proteins of unknown active site structure. The probes include most of the possible isomers of the phenyl, naphthyl, and biphenyl ring systems. A selection of the probes has been shown to react with the model protein $P450_{cam}$ to give (arylazido)iron (Fe−ArN_3) complexes, a prerequisite for subsequent affinity labeling of the protein.

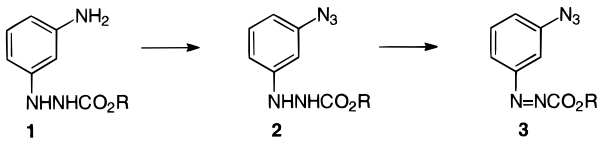
The study of membrane-bound cytochrome P450 enzymes has been hindered by a lack of the structural information that can usually be obtained by X-ray crystallography or nuclear magnetic resonance. In an effort to bridge this gap, we have introduced the use of aryldiazenes to probe the active site of heme-containing proteins.[1−3] Aryldiazenes react with hemoproteins to form stable σ-bonded Fe−aryl complexes.[4,5] In the case of the cytochromes P450, which have a thiolate ligand, this complex can be oxidized in the intact active site resulting in a regioselective shift of the aryl group to one or more of the pyrole nitrogens of the heme (Scheme 1).[6,7] The direction of the shift is primarily governed by the steric encumbrance within the active site. Extraction, HPLC separation, and quantitation of the *N*-arylprotoporphyrin IX adducts can thus be used to obtain a low-resolution topological map of the active site. This method is limited, however, since it does not give information on the residues that interact with the aryl probes. We have undertaken, therefore, to prepare aryldiazenes bearing a photoreactive group that can cross-link to the protein which, after oxidative shift of the aryl group to the heme, would result in covalent attachment of the heme to the protein. We have recently validated the potential of this approach by successfully cross-linking His64 to the heme in myoglobin using (*m*-azidophenyl)diazene or (*p*-azidophenyl)diazene,[8] and we here report the synthesis of a series of (azidoaryl)diazenecarboxylate esters as potential general probes for active site mapping of heme-containing proteins.

Aryldiazenes are moderately unstable and are best prepared immediately *prior* to use by base hydrolysis of aryldiazenecarboxylate esters.[9] The synthetic scheme for these compounds was therefore designed with carboxylate ester group as the protective group for the hydrazine (see below). Initial attempts to prepare azide **2** (R = *t*-Bu) by diazotation of amine **1** in diluted HCl at 4 °C and subsequent nucleophilic displacement by NaN_3 (Scheme 2) gave a complex mixture containing less than 3% of the desired product. Application of the method developed by Melhado and Leonard,[10] in which diluted HCl was replaced by 80% AcOH, still gave a mixture but **2** and **3** were obtained in 15% and 14% yield, respectively. By adding NaN_3 prior to $NaNO_2$ and carrying out the reaction at room temperature, we obtained azides **2** and **3** in 10 and 40% yield, respectively, along with unreacted amine **1** as the sole products of the reaction. This latest result clearly indicated that (a) addition of NaN_3 prior to diazotation diminishes the number of products formed and (b) $NaNO_2$ oxidizes the hydrazine **2** to the diazene **3**, and thus excess reagent is required to carry the reaction to completion. To the best of our knowledge, oxidation of acylhydrazines by sodium nitrite has not been previously reported. Following these observations, we devised a simple and rapid method for obtaining (azidoaryl)diazenes from the corresponding (nitroaryl)-hydrazines (Scheme 3). In this scheme the reaction is carried out in AcOH and the intermediate amine is not isolated but is immediately converted to the final product by addition of NaN_3 and 2−3 equiv of $NaNO_2$. A range of compounds was synthesized using this methodology (Figure 1). Table 1 gives the yields obtained for the synthesis of a series of phenyl, biphenyl, and naphthyl

Scheme 1



Scheme 2



1 **2** **3**

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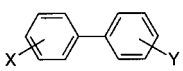
4 **a** X = $NHNH_2$, Y = 3-NO_2
b X = $NHNH_2$, Y = 4-NO_2

5 **a** X = $NHNHCO_2Et$, Y = 3-NO_2
b X = $NHNHCO_2Et$, Y = 4-NO_2

6 **a** X = $N{=}NCO_2Et$, Y = 3-N_3
b X = $N{=}NCO_2Et$, Y = 4-N_3

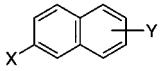
7 **a** X = H
b X = F



8 **a** X = 4-NH_2, Y = 4-NO_2
b X = 4-NH_2, Y = 3-NO_2
c X = 3-NH_2, Y = 4-NO_2
d X = 3-NH_2, Y = 3-NO_2

9 **a** X = 4-$NHNHCO_2Et$, Y = 4-NO_2
b X = 4-$NHNHCO_2Et$, Y = 3-NO_2
c X = 3-$NHNHCO_2Et$, Y = 4-NO_2
d X = 3-$NHNHCO_2Et$, Y = 3-NO_2

10 **a** X = 4-$N{=}NCO_2Et$, Y = 4-N_3
b X = 4-$N{=}NCO_2Et$, Y = 3-N_3
c X = 3-$N{=}NCO_2Et$, Y = 4-N_3
d X = 3-$N{=}NCO_2Et$, Y = 3-N_3



11 **a** X = NH_2, Y = 5-NO_2
b X = NH_2, Y = 6-NO_2
c X = NH_2, Y = 7-NO_2
d X = NH_2, Y = 8-NO_2

12 **a** X = $NHNHCO_2Et$, Y = 5-NO_2
b X = $NHNHCO_2Et$, Y = 6-NO_2
c X = $NHNHCO_2Et$, Y = 7-NO_2
d X = $NHNHCO_2Et$, Y = 8-NO_2

13 **a** X = $N{=}NCO_2Et$, Y = 5-N_3
b X = $N{=}NCO_2Et$, Y = 6-N_3
c X = $N{=}NCO_2Et$, Y = 7-N_3
d X = $N{=}NCO_2Et$, Y = 8-N_3

Figure 1. Structures of the starting materials, intermediates, and products.

Scheme 3

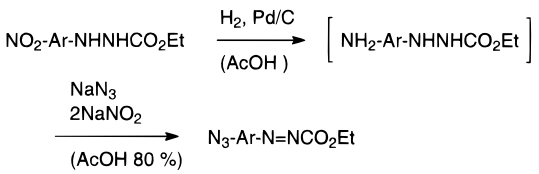


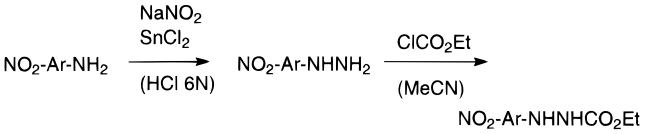
Table 1. Preparation of (Nitroaryl)hydrazines and Conversion to (Azidoaryl)diazene

from	$NO_2ArNHNHCO_2Et$	yield (%)	$N_3ArN{=}NCO_2Et$	yield (%)
4a	**5a**	90	**6a**	68
4b	**5b**	98	**6b**	72
14a	**15a**	5[a]	**7a**	63
14b	**15b**	18[a]	**7b**	61
8a	**9a**	78[b]	**10a**	77
8b	**9b**	70[b]	**10b**	92
8c	**9c**	17[b]	**10c**	91
8d	**9d**	1[b]	**10d**	86
11a	**12a**	57[b]	**13a**	52
11b	**12b**	85[b]	**13b**	41
11c	**12c**	53[b]	**13c**	55
11d	**12d**	3[b]	**13d**	72

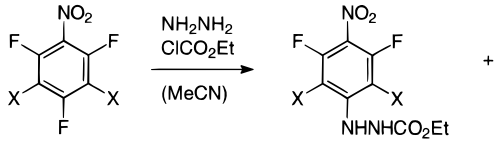
[a] Scheme 5. [b] Scheme 4.

azido−diazene probes. The procedure appears to be of wide scope, and yields in the series of compound that were examined were high irrespective of the structure of the starting compound. The (nitrobiphenyl)hydrazines **9a**−**d** and (nitronaphthyl)hydrazines **12a**−**d** were prepared from the corresponding (nitroaryl)amines **8a**−**d** and **11a**−**d**, respectively, by diazotation in 6 N HCl followed by reduction with $SnCl_2$ and derivatization with ClCOOEt (Scheme 4, Table 1). In the case of the naphthyl derivatives, temperature control was found to be critical during diazotation and reduction, and no desired product

Scheme 4

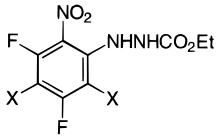


Scheme 5



14 **a** X = H
b X = F

15 **a** X = H (5 %)
b X = F (18 %)



16 **a** X = H (70 %)
b X = F (37 %)

was detected when the reactions were out carried above 0 °C.

A possible limitation in the use of aryl azide for photoaffinity labeling is the short lifetime of the photogenerated singlet nitrene and ring expansion to a didehydroazepine.[11a−d] Substitution with fluorine at positions 2 and 6 relative to the azido group raises the barrier of ring expansion to 8 kcal/mol and increases the lifetime of the nitrene from 10−100 ps to 10−100 ns at room temperature.[12,13] The fluorinated arylhydrazines **15a**,**b** were therefore also prepared by reacting hydrazine with 2,4,6-trifluoronitrobenzene (**14a**) and pentafluoronitrobenzene (**14b**), respectively, followed by immediate reaction of the arylhydrazine with ClCOOEt to prevent further reaction (Scheme 5). Addition of hydrazine ortho to the nitro group predominated in both cases. The yields of the desired *para* adduct could not be improved by varying temperature or solvent or by replacing NH_2NH_2 with NH_2NHLi or NH_2NHNa.[14] Conversion of hydrazines **15a,b** to the corresponding (azidoaryl)diazene did not suffer from the fluorine substitution, and **7a,b** were obtained in high yields (Table 1).

Figure 2 shows the UV spectra of the reactions of the diazenes generated from base hydrolysis of **6a**, **10a**, and **13b** with camphor-bound cytochrome $P450_{cam}$. The initial Soret absorbance at 395 nm is lost and is replaced by a less intense band at 478 nm characteristic for thiol-ligated Fe−aryl complexes.[5] These results show that addition of the azido group did not hamper the formation of the Fe−aryl complexes, a prerequisite if they are to be used to photolabel hemoprotein active sites. The reaction with 4′-azido-4-biphenyldiazene is slightly slower (Figure 2B), a not unexpected result given the larger size of the probe and the fact that the resulting aryl−iron

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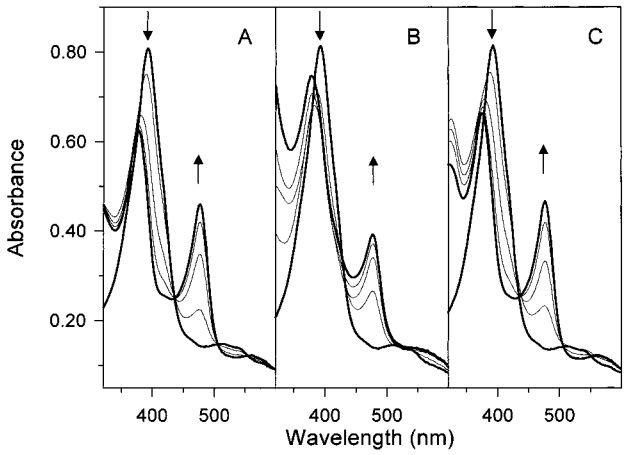

Figure 2. Reaction of $P450_{cam}$ with (A) (*m*-azidophenyl)-diazene, (B) (4-(4-azidophenyl)phenyl)diazene, and (C) (6-azido-2-naphthyl)diazene. The original Soret band of camphor-bound $P450_{cam}$ (395 nm) is replaced by a band at 480 nm, typical of Fe−aryl complexes.[1]

complex extends to a height of approximatively 10 Å above the heme plane.

In conclusion, we report the synthesis of a family of chemical probes for mapping the active sites of heme-containing proteins. The probes, which form (azidoaryl)-iron complexes with the heme iron atom, place a photo-activatable moiety at defined distances and orientations with respect to the heme plane. Photocross-linking to the protein should provide, as found in a test case,[8] important information on the residues that define the active site and their topological arrangement.

Experimental Section

CAUTION: *All naphthalene amino products are suspected carcinogens and should be handled accordingly.*

General Comments. Melting points are uncorrected. ^1H and ^{13}C NMR spectra were recorded at 300 MHz using TMS as a standard. IR spectra (cm^{-1}) were obtained in KBr on a FT spectrophotometer. Mass spectra were obtained by at the University of California Mass Spectrometry Facility. Starting materials were prepared according to standard procedures. All reagents were purchased from chemical suppliers and used without further purification. Chromatography was carried out with Merck 60 (230−600 mesh) silica gel. Cytochrome $P450_{cam}$ was expressed and purified as previously described.[5]

Iron−Aryl Complex Formation with $P450_{cam}$. The diazenecarboxylate esters (0.5 mg) were dissolved in 40 μL of methanol and hydrolyzed by addition of 2 μL of 2 N NaOH. The diazene solution thus formed (2 μL) was then added to 500 μL of a solution of $P450_{cam}$ (10 μM in 50 mM phosphate buffer pH 7.3).

Preparation of (Nitrophenyl)hydrazinecarboxylate Esters. The (nitrophenyl)hydrazines were dissolved in 100 mL of acetonitrile at room temperature. Pyridine (1.1 equiv) and ethyl chloroformate (1.1 equiv) were added successively, and the mixture was stirred for 10 min. The products were purified by column chromatography (50:50 hexanes/ethyl acetate).

2-(3-Nitrophenyl)hydrazinecarboxylate, ethyl ester (5a): obtained as a yellow solid (98%), mp 106−108 °C; ^1H NMR δ ($CDCl_3$) 1.286 (m, 3H), 4.213 (q, J = 6.9 Hz, 2H), 6.468 (s, 1H), 6.863 (s, 1H), 7.059 (dd, J = 8.1, 1.8 Hz, 1H), 7.298 (t, J = 8.1 Hz, 1H), 7.611 (d, J = 1.8 Hz, 1H), 7.658 (d, J = 8.1 Hz, 1H); ^{13}C NMR δ ($CDCl_3$) 14.371, 62.316, 107.560, 115.254, 118.585, 129.767, 149.176, 149.351, 157.071; IR ν (KBr) 3369, 3297, 1696, 1528, 1350; HRMS (EI) calcd for $C_9H_{11}N_3O_4$ (M^+) *m/e* 225.0749, found *m/e* 225.0755.

2-(4-Nitrophenyl)hydrazinecarboxylate, ethyl ester (5b): obtained as a pale brown solid (90%), mp 195−196 °C (dec); ^1H NMR δ (DMSO-d_6) 1.193 (t, J = 6.9 Hz, 3H), 4.072 (q, J = 6.9 Hz, 2H), 6.725 (d, J = 9.0 Hz, 2H), 8.064 (d, J = 9.0 Hz, 2H), 8.961 (s, 1H), 9.379 (s, 1H); ^{13}C NMR δ (DMSO-d_6) 14.495, 60.713, 110.259, 125.892, 138.038, 154.997, 156.388; IR ν (KBr) 3342, 3301, 1682, 1506, 1332; HRMS (EI) calcd for $C_9H_{11}N_3O_4$ (M^+) *m/e* 225.0749, found *m/e* 225.0753.

Procedure for the Preparation of o-Fluorinated 2-(Nitrophenyl)hydrazinecarboxylate Esters. (2,4,6-Trifluoronitro)benzene or pentafluoronitrobenzene (5−30 mmol) was dissolved in 50−100 mL of acetonitrile and pyridine (2.2 equiv). Hydrazine (1.1 equiv) was added and the mixture stirred for an additional 10 min. After addition of ethyl chloroformate (1.1 equiv), the mixture was evaporated and the residue purified by column chromatography.

2-(3,5-Difluoro-4-nitrophenyl)hydrazinecarboxylate, ethyl ester (15a): obtained as a beige solid (5%), mp 118−120 °C; ^1H NMR δ ($CDCl_3$) 1.292 (t, J = 7.2 Hz, 3H), 4.210 (q, J = 7.2 Hz, 2H), 6.458 (d, J_{H-F} = 11.1 Hz, 2H), 6.845 (s, 1H), 6.950 (s, 1H); ^{13}C NMR δ (acetone-d_6) 14.746, 62.220, 95.664 (d, J_{C-F} = 100.8 Hz), 121.348 (t, J_{C-F} = 39.5 Hz), 155.420 (t, J_{C-F} = 52.8 Hz), 157,145, 157.860 (d, J_{C-F} = 1027.5 Hz); IR ν (KBr) 3362, 3289, 1685, 1531, 1344; HRMS (EI) calcd for $C_9H_9F_2N_3O_4$ (M^+) *m/e* 261.0561, found *m/e* 261.0565.

2-(3,5-Difluoro-2-nitrophenyl)hydrazinecarboxylate, ethyl ester (16a): obtained as a yellow solid (70%), mp 90−92 °C; ^1H NMR δ (acetone-d_6) 1.235 (t, J = 6.9 Hz, 3H), 4.158 (q, J = 6.9 Hz, 2H), 6.668 (ddd, J = 2.4, 8.7, 11.4 Hz, 1H), 6.801 (ddd, J = 2.1, 2.4, 11.7 Hz, 1H), 8.586 (s, 1H), 8.789 (s, 1H); ^{13}C NMR δ (acetone-d_6) 14.708, 62.360, 95.738 (t, J_{C-F} = 105.9 Hz), 97.028 (d, J_{C-F} = 117.0 Hz) 122.784 (d, J_{C-F} = 38.7 Hz), 148.814 (d, J_{C-F} = 55.2 Hz), 157.173, 159.106 (dd, J_{C-F} = 64.4, 1035.6 Hz), 166.178 (dd J_{C-F} = 64.4, 1035.6 Hz); IR ν (KBr) 3319, 1698, 1585, 1341; HRMS (EI) calcd for $C_9H_9F_2N_3O_4$ (M^+) *m/e* 261.0561, found *m/e* 261.0564.

2-(2,3,5,6-Tetrafluoro-4-nitrophenyl)hydrazinecarboxylate, ethyl ester (15b): obtained as a beige solid (18%), mp 135−136 °C; ^1H NMR δ ($CDCl_3$) 1.287 (t, J = 7.2 Hz, 3H), 4.127 (q, J = 7.2 Hz, 2H), 6.245 (s, 1H), 6.772 (s, 1H); ^{13}C NMR δ (acetone-d_6) 14.729, 62.326, 121.692 (t, J_{C-F} = 39.6 Hz), 134.225, 137.144 (dd, J_{C-F} = 54.9, 980.4 Hz), 143.044 (dd, J_{C-F} = 53.7, 1035.0 Hz), 157.476; IR ν (KBr) 3333, 3308, 1684, 1560, 1345; HRMS (EI) calcd for $C_9H_7F_4N_3O_4$ (M^+) *m/e* 297.0373, found *m/e* 297.0362.

2-(2,3,4,5-Tetrafluoro-6-nitrophenyl)hydrazinecarboxylate, ethyl ester (16b): obtained as a yellow solid (37%), mp 106−107 °C; ^1H NMR δ ($CDCl_3$, tms) 1.179 (t, J = 7.2 Hz, 3H), 4.078 (q, J = 7.2 Hz, 2H), 7.854 (s, 1H), 8.658 (s, 1H); ^{13}C NMR δ (acetone-d_6) 14.660, 62.433, 126.843 (d, J_{C-F} = 42.9 Hz), 131.135 (d, J_{C-F} = 42.9 Hz), 135.105 (td, J_{C-F} = 57.3, 982.2 Hz), 139.068 (dd, J_{C-F} = 46.8, 1030.8 Hz), 142.463 (dd, J_{C-F} = 49.8, 1014.3 Hz), 143.889 (td, J_{C-F} = 55.8, 1016.1 Hz), 157.943; IR ν (KBr) 3304, 1692, 1557, 1363; HRMS (EI) calcd for $C_9H_7F_4N_3O_4$ (M^+) *m/e* 297.0373, found *m/e* 297.0361.

General Procedure for the Preparations of (Nitroaryl)-hydrazinecarboxylic Acid, Ethyl Esters from the Corresponding Amine. To a suspension of 100−300 mg of the starting amine in 15 mL of 6 N HCl at −4 °C was added sodium nitrite (1.1 equiv in 0.2−0.5 mL of water). The mixture was allowed to react for 15 min before addition of stannous chloride (2.5 equiv in 0.4−1 mL of 12 N HCl). Stirring and cooling were maintained for another 15 min. The mixture was then neutralized with saturated sodium carbonate and extracted three times with ethyl acetate. The organic layers were combined, washed with brine, dried over magnesium sulfate, and evaporated to dryness. The residue was immediately redissolved in 15 mL acetonitrile, and pyridine and ethyl chloroformate were added in 1.1 equiv each. The mixture was stirred for 10 min, and after removal of the solvent under vacuum, the residue was purified by column chromatography.

2-(4-(4-Nitrophenyl)phenyl)hydrazinecarboxylate, ethyl ester (9a): obtained as an orange solid (59%), mp 210−212 °C; ^1H NMR δ (CD_3CN) 1.223 (t, J = 6.9 Hz, 3H), 4.113 (q, J = 6.9 Hz, 2H), 6.572 (s, 1H), 6.882 (d, J = 8.7 Hz, 2H),

7.393 (s, 1H), 7.599 (d, J = 8.7 Hz, 2H), 7.780 (d, J = 8.7 Hz, 2H), 8.228 (d, J = 8.7 Hz, 2H); ^{13}C NMR δ (DMSO-d_6) 14.557, 60.348, 112.003, 124.043, 126.129, 127.127, 127.943, 145.337, 146.803, 150.464, 156.789; IR ν (KBr) 3318, 1684, 1586, 1341; HRMS (EI) calcd for $C_{15}H_{15}N_3O_4$ (M^+) *m/e* 301.1063, found *m/e* 301.1058.

2-(4-(3-Nitrophenyl)phenyl)hydrazinecarboxylate, ethyl ester (9b): obtained as a yellow solid (76%), mp 155−156 °C; ^{1}H NMR δ ($CDCl_3$) 1.300 (t, J = 7.2 Hz, 3H), 4.226 (q, J = 7.2 Hz, 2H), 5.971 (s, 1H), 6.638 (s, 1H), 6.935 (d, J = 8.1 Hz, 2H), 7.509 (d, J = 8.1 Hz, 2H), 7.546 (t, J = 8.4 Hz, 1 H), 7.838 (d, J = 8.4 Hz, 1H), 8.121 (d, J = 8.4 Hz, 1H), 8.375 (s, 1H); ^{13}C NMR δ (acetone-d_6) 14.911, 61.544, 113.652, 121.101, 121.513, 128.499, 129.991, 130.828, 132.952, 143.619, 149.778, 151.092, 157.80; IR ν (KBr) 3395, 3245, 1708, 1515, 1345; HRMS (EI) calcd for $C_{15}H_{15}N_3O_4$ (M^+) *m/e* 301.1063, found *m/e* 301.1062.

2-(3-(4-Nitrophenyl)phenyl)hydrazinecarboxylate, ethyl ester (9c): obtained as a yellow solid (17%), mp 127−128 °C; ^{1}H NMR δ (CD_3CN) 1.278 (t, J = 6.9 Hz, 3H), 4.234 (q, J = 6.9 Hz, 2H), 6.093 (s, 1H), 6.739 (s, 1H), 6.882 (d, J = 7.8 Hz, 1H), 7.019 (s, 1H), 7.087 (d, J = 7.8 Hz, 1H), 7.314 (t, J = 7.8 Hz, 1H), 7.634 (d, J = 8.7 Hz, 2H), 8.231 (d, J = 8.7 Hz, 2H); ^{13}C NMR δ ($CDCl_3$) 14.447, 62.029, 111.676, 113.359, 119.887, 123.860, 127.696, 129.896, 139.717, 146.968, 147.541, 148.783, 157.171; IR ν (KBr) 3362, 3296, 1693, 1511, 1349; HRMS (EI) calcd for $C_{15}H_{15}N_3O_4$ (M^+) *m/e* 301.1063, found *m/e* 301.1061.

2-(3-(3-Nitrophenyl)phenyl)hydrazinecarboxylate, ethyl ester (9d): obtained as an orange solid (19%), mp 119−120 °C. ^{1}H NMR δ ($CDCl_3$) 1.268 (t, J = 6.9 Hz, 3H), 4.203 (q, J = 6.9 Hz, 2H), 6.153 (s, 1H), 6.831 (s, 1H), 6.845 (d, J = 7.8 Hz, 1H), 6.994 (s, 1H), 7.050 (d, J = 8.1 Hz, 1H), 7.278 (t, J = 7.8 Hz, 1H), 7.523 (t, J = 8.1 Hz, 1H), 7.792 (d, J = 7.8 Hz, 1H), 8.135 (d, J = 8.1 Hz, 1H), 8.316 (s, 1H); ^{13}C NMR δ ($CDCl_3$) 14.405, 61.976, 111.413, 112.913, 119.491, 121.744, 121.890, 129.433, 129.839, 139.496, 142.690, 148.445, 148.800, 157.243; IR ν (KBr) 3318, 1681, 1522, 1345; HRMS (EI) calcd for $C_{15}H_{15}N_3O_4$ (M^+) *m/e* 301.1063, found *m/e* 301.1060.

2-(5-Nitro-2-naphthyl)hydrazinecarboxylate, ethyl ester (12a): obtained as a yellow solid (57%), mp 145−146 °C; ^{1}H NMR δ (acetone-d_6) 1.303 (t, J = 7.2 Hz, 3H), 4.234 (q, J = 7.2 Hz, 2H), 6.307 (s, 1H), 6.750 (s, 1H), 7.157 (s, 1H), 7.186 (d, J = 9.3 Hz, 1H), 7.393 (t, J = 7.5 Hz, 1H), 7.840 (d, J = 7.5 Hz, 1H), 7.989 (d, J = 7.5 Hz, 1H), 8.369 (d, J = 8.369 Hz, 1H); ^{13}C NMR δ (CD_2Cl_2 + 5% CD_3OD) 10.912, 58.633, 103.112, 115.193, 116.638, 117.573, 120.752, 121.232, 129.602, 132.737, 143.128, 143.638, 153.908; IR ν (KBr) 3335, 3303, 1697, 1514, 1334; HRMS (EI) calcd for $C_{13}H_{13}N_3O_4$ (M^+) *m/e* 275.0906, found *m/e* 275.0908.

2-(6-Nitro-2-naphthyl)hydrazinecarboxylate, ethyl ester (12b): obtained as yellow solid (85%), mp 239−242 °C (dec); ^{1}H NMR δ (DMSO-d_6) 1.241 (t, J = 6.9 Hz, 3H), 4.112 (q, J = 6.9 Hz, 2H), 7.022 (s, 1H), 7.196 (dd, J = 0.9, 8.7 Hz, 1H), 7.834 (d, J = 9.3 Hz, 1H), 8.051 (d, J = 8.7 Hz, 1H), 8.071 (dd, J = 1.8, 8.7 Hz, 1H), 8.636 (s, 1H), 8.777 (d, J = 0.9 Hz, 1H), 9.340 (s, 1H); ^{13}C NMR δ (DMSO-d_6) 14.498, 60.524, 103.280, 117.251, 119.534, 124.780, 125.309, 126.904, 131.419, 137.774, 141.598, 150.842, 156.605; IR ν (KBr) 3317, 1683, 1624, 1330; HRMS (EI) calcd for $C_{13}H_{13}N_3O_4$ (M^+) *m/e* 275.0906, found *m/e* 275.0912.

2-(7-Nitro-2-naphthyl)hydrazinecarboxylate, ethyl ester (12c): obtained as an orange solid (53%), mp 154−155 °C; ^{1}H NMR δ (CD_3CN) 1.217 (br, 3H), 4.113 (q, J = 7.2 Hz, 2H), 6.782 (s, 1H), 7.21 (m, 1H), 7.234 (s, 1H), 7.494 (s, 1H), 7.792 (d, J = 9.6 Hz, 1H), 7.831 (d, J = 9.0 Hz, 1H), 7.896 (dd, J = 2.1 Hz, J = 8.7 Hz, 1H), 8.555 (d, J = 2.1 Hz, 1H); ^{13}C NMR δ (CD_3CN) 14.912, 62.332, 107.821, 116.770, 118.276, 120.260, 123.360, 126.760, 123.521, 130.031, 130.247, 131.884, 134.478, 147.196, 149.514, 157.952; IR ν (KBr) 3371, 3310, 1696, 1344; HRMS (EI) calcd for $C_{13}H_{13}N_3O_4$ (M^+) *m/e* 275.0906, found *m/e* 275.0902.

2-(8-Nitro-2-naphthyl)hydrazinecarboxylate, ethyl ester (12d): obtained as an orange solid (55%), mp 141−142 °C; ^{1}H NMR δ ($CDCl_3$) 1.298 (t, J = 6.9 Hz, 3H), 4.225 (q, J = 6.9 Hz, 2H), 6.466 (s, 1H), 6.852 (s, 1H), 7.076 (d, J = 8.7 Hz, 1H), 7.256 (t, J = 7.8 Hz, 1H), 7.667 (d, J = 8.7 Hz, 1H), 7.898 (d, J = 7.8 Hz, 1H), 7.911 (s, 1H), 8.213 (d, J = 7.8 Hz, 1H); ^{13}C NMR δ ($CDCl_3$) 14.450, 62.258, 101.762, 116.450, 120.929, 125.268, 130.122, 130.233, 134.793, 144.634, 149.120, 157.071; IR ν (KBr) 3352, 3308, 1688, 1632, 1517; HRMS (EI) calcd for $C_{13}H_{13}N_3O_4$ (M^+) *m/e* 275.0906, found *m/e* 275.0901.

General Procedure for the Preparation of (Azidoaryl)-diazenes. All azides were stored foil-wrapped at −20 °C and showed no decomposition by TLC for 1−2 years. The parent nitro compound was hydrogenated in 20 mL of acetic acid with 10% Pd/C in a Parr apparatus. Following reduction, the solution was filtered and water was added to adjust the solvent to 80% acetic acid. The flask was wrapped in aluminum foil, and NaN_3 and $NaNO_2$, in 0.1 mg/μL solutions, were added successively in 1−2 and 2−3 eq, respectively. The reaction mixture was stirred at room temperature for 4−16 h and monitored by TLC. The mixture was then evaporated to dryness, redissolved in CH_2Cl_2, and purified by column chromatography (CH_2Cl_2).

(3-Azidophenyl)diazenecarboxylate, ethyl ester (6a): obtained as an orange oil (68%); ^{1}H NMR δ ($CDCl_3$) 1.496 (t, J = 6.9 Hz, 3H), 4.523 (q, J = 6.9 Hz, 2H), 7.210 (dd, J = 8.1, 1.8 Hz, 1H), 7.508 (t, J = 8.1 Hz, 1H), 7.556 (d, J = 1.8 Hz, 1H), 7.728 (d, J = 8.1 Hz, 1H); ^{13}C NMR δ ($CDCl_3$) 13.976, 64.414, 112.413, 121.249, 123.821, 130.385, 141.533, 152.493, 161.810; IR ν (KBr) 2112, 1759; HRMS (EI) calcd for $C_9H_9N_5O_2$ (M^+) *m/e* 219.0756, found *m/e* 219.0749.

(4-Azidophenyl)diazenecarboxylate, ethyl ester (6b): obtained as an orange oil (72%); ^{1}H NMR δ ($CDCl_3$) 1.474 (t, J = 7.2 Hz, 3H), 1.491 (t, J = 7.2 Hz, 3H), 4.522 (q, J = 7.2 Hz, 2H), 4.555 (q, J = 7.2 Hz, 2H), 7.163 (d, J = 8.7 Hz, 2H), 7.972 (d, J = 8.7 Hz, 2H), 8.057 (d, J = 9.0 Hz, 2H), 8.405 (d, J = 9.0 Hz, 2H); ^{13}C NMR δ ($CDCl_3$) 14.128, 64.425, 119.683, 125.750, 145.798, 148.635, 161.946; IR ν (KBr) 2219, 1755; HRMS (EI) calcd for $C_9H_9N_5O_2$ (M^+) *m/e* 219.0756, found *m/e* 219.0765.

4-Azido-3,5-difluorophenyldiazenecarboxylate, Ethyl Ester (7a): obtained as an orange oil (63%); ^{1}H NMR δ ($CDCl_3$) 1.470 (t, J = 7.2 Hz, 3H), 4.523 (t, J = 7.2 Hz, 2H), 7.562 (d, J_{H-F} = 8.7 Hz, 0.4H), 7.660 (d, J = 8.1 Hz, 1.6H); ^{13}C NMR δ ($CDCl_3$) 14.057, 64.712, 107.868 (d, J_{C-F} = 96.6 Hz), 122.804 (t, J_{C-F} = 57.6 Hz), 147.331 (t, J_{C-F} = 29.7 Hz), 155.523 (dd, J_{C-F} = 13.3, 1008.6 Hz), 161.439; IR ν (KBr) 2140, 1768; HRMS (EI) calcd for $C_9H_8F_2N_5O_2$ (M^+ + 1) *m/e* 256.0646, found *m/e* 256.0650.

(4-Azido-2,3,5,6-tetrafluorophenyl)diazenecarboxylate, ethyl ester (7b): obtained as an orange oil (61%); ^{1}H NMR δ ($CDCl_3$) 1.474 (t, J = 7.2 Hz, 3H), 4.530 (q, J = 7.2 Hz, 2H); ^{13}C NMR δ ($CDCl_3$) 14.095, 64.059, 109.057 (t, J_{C-F} = 91.8 Hz), 124.364 (t, J_{C-F} = 48.0 Hz), 140.632 (ddd, J_{C-F} = 28.2, 72.6, 992.7 Hz), 141.430 (ddd, J_{C-F} = 14.7, 53.4, 1060.2 Hz), 160.911; IR ν (KBr) 2157, 1762; HRMS (EI) calcd for $C_7F_4N_5O$ ($M^+ - C_2H_5O$) *m/e* 246.0039, found *m/e* 246.0038.

(4-(4-Azidophenyl)phenyl)diazenecarboxylate, ethyl ester (10a): obtained as an orange oil (77%); ^{1}H NMR δ ($CDCl_3$) 1.485 (t, J = 6.9 Hz, 3H), 4.536 (q, J = 6.9 Hz, 2H), 7.132 (d, J = 8.7 Hz, 2H), 7.650 (d, J = 8.7 Hz, 2H), 7.721 (d, J = 8.7 Hz, 2H), 8.015 (d, J = 8.7 Hz, 2H); ^{13}C NMR δ ($CDCl_3$) 14.165, 64.423, 119.613, 124.453, 127.526, 128.892, 136.173, 140.492, 145.492, 150.737, 162.111; IR ν (KBr) 2102, 1755; HRMS (EI) calcd for $C_{15}H_{13}N_5O_2$ (M^+) *m/e* 295.1069, found *m/e* 295.1057.

(4-(3-Azidophenyl)phenyl)diazenecarboxylate, ethyl ester (10b): obtained as an orange oil (92%); ^{1}H NMR δ ($CDCl_3$) 1.487 (t, J = 7.2 Hz, 3H), 4.539 (q, J = 7.2 Hz, 2H), 7.091 (d, J = 7.5 Hz, 2H), 7.272 (d, J = 1.2 Hz, 1H), 7.729 (m, 2H), 8.022 (d, J = 8.7 Hz, 2H); ^{13}C NMR δ ($CDCl_3$) 14.128, 64.435, 117.789, 118.808, 123.812, 124.346, 127.915, 130.303, 140.816, 141.359, 145.372, 150.974, 162.070; IR ν (KBr) 2105, 1752; HRMS (EI) calcd for $C_{15}H_{13}N_5O_2$ (M^+) *m/e* 295.1069, found *m/e* 295.1059.

(3-(4-Azidophenyl)phenyl)diazenecarboxylate, ethyl ester (10c). Obtained as an orange oil (91%); ^{1}H NMR δ ($CDCl_3$) 1.481 (t, J = 6.9 Hz, 3H), 4.535 (q, J = 6.9 Hz, 2H),

7.110 (d, J = 8.4 Hz, 2H), 7.585 (t, J = 7.8 Hz, 1H), 7.614 (d, J = 8.4 Hz, 2H), 7.773 (d, J = 8.1 Hz, 1H), 7.902 (d, J = 8.1 Hz, 1H), 8.122 (s, 1H); ^{13}C NMR δ ($CDCl_3$) 14.1112, 64.470, 119.530, 121.780, 122.552, 128.382, 129.745, 131.814, 136.210, 139.950, 141.355, 152.050, 162.043; IR ν (KBr) 2124, 1757; HRMS (EI) calcd for $C_{15}H_{13}N_5O_2$ (M^+) *m/e* 295.1069, found *m/e* 295.1066.

(3-(3-Azidophenyl)phenyl)diazenecarboxylate, ethyl ester (10d): obtained as an orange oil (86%); ^{1}H NMR δ ($CDCl_3$) 1.484 (t, J = 7.2 Hz, 3H), 4.540 (q, J = 7.2 Hz, 2H), 7.056 (d, J = 6.9 Hz, 1H), 7.252 (s, 1H), 7.389 (d, J = 7.5 Hz, 1H), 7.443 (t, J = 7.5 Hz, 1H), 7.604 (t, J = 7.8 Hz, 1H), 7.786 (d, J = 7.5 Hz, 1H), 7.933 (d, J = 7.8 Hz, 1H), 8.134 (s, 1H); ^{13}C NMR δ ($CDCl_3$) 14.134, 64.538, 117.666, 118.449, 122.214, 122.963, 123.678, 129.812, 130.293, 132.166, 140.759, 141.389, 151.990, 162.022; IR ν (KBr) 2109, 1756; HRMS (EI) calcd for $C_{15}H_{13}N_5O_2$ (M^+) *m/e* 295.1069, found *m/e* 295.1078.

(5-Azido-2-naphthyl)diazenecarboxylate, ethyl ester (13a): obtained as a yellow solid (52%), mp 68−69 °C; ^{1}H NMR δ ($CDCl_3$) 1.496 (t, J = 7.2 Hz, 3H), 4.552 (q, J = 7.2 Hz, 2H), 7.387 (d, J = 7.5 Hz, 1H), 7.578 (7, J = 7.5 Hz, 1H), 7.793 (d, J = 7.5 Hz, 1H), 7.906 (d, J = 9.0 Hz, 1H), 8.169 (d, J = 9.0 Hz, 1H), 8.547 (s, 1H); ^{13}C NMR δ ($CDCl_3$) 14.122, 64.423, 116.637, 115.918, 124.287, 126.382, 127.108, 128.616, 130.781, 134.170, 137.070, 149.907, 162.139; IR ν (KBr) 3113, 1750; HRMS (EI) calcd for $C_{13}H_{11}N_5O_2$ (M^+) *m/e* 269.0913, found *m/e* 269.0907.

(6-Azido-2-naphthyl)diazenecarboxylate, ethyl ester (13b): obtained as an orange solid (41%), mp 66−68 °C; ^{1}H NMR δ ($CDCl_3$) 1.491 (t, J = 7.2 Hz, 3H), 4.546 (q, J = 7.2 Hz, 2H), 7.231 (dd, J = 1.8, 8.7 Hz, 1H), 7.466 (s, 1H), 7.777 (d, J = 8.7 Hz, 1H), 7.928 (dd, J = 1.8, 8.7 Hz, 1H), 7.988 (d, J = 8.7 Hz, 1H), 8.528 (s, 1H); ^{13}C NMR δ ($CDCl_3$) 14.164, 64.385, 115.965, 116.952, 120.001, 128.372, 130.470, 131.607, 131.932, 136.856, 140.913, 148.966, 162.153; IR ν (KBr) 2108, 1747; HRMS (EI) calcd for $C_{13}H_{11}N_5O_2$ (M^+) *m/e* 269.0913, found *m/e* 269.0904.

(7-Azido-2-naphthyl)diazenecarboxylate, ethyl ester (13c): obtained as an orange solid (55%), mp 63−64 °C; ^{1}H NMR δ (CD_2Cl_2) 1.452 (t, J = 7.2 Hz, 3H), 4.500 (q, J = 7.2 Hz, 2H), 7.274 (dd, J = 9.0 Hz, J = 2.1 Hz, 1H), 7.604 (d, J = 2.1 Hz, 1H), 7.786 (dd, J = 9.0 Hz, J = 2.1 Hz, 1H), 7.858 (d, J = 8.7 Hz, 1H), 7.877 (d, J = 8.7 Hz, 1H), 8.424 (s, 1H); ^{13}C NMR δ (CD_2Cl_2) 14.328, 64.821, 115.212, 117.799, 121.936, 129.795, 130.314, 130.517, 133.724, 134.191, 139.518, 150.396, 162.624; IR ν (KBr) 2115, 1751; HRMS (EI) calcd for $C_{13}H_{11}N_5O_2$ (M^+) *m/e* 269.0913, found *m/e* 269.0907.

(8-Azido-2-naphthyl)diazenecarboxylate, ethyl ester (13d): obtained as an orange solid (72%), mp 83−85 °C; ^{1}H NMR δ ($CDCl_3$) 1.496 (t, J = 7.2 Hz, 3H), 4.508 (q, J = 7.2 Hz, 2H), 7.332 (d, J = 7.2 Hz, 1H), 7.601 (t, J = 7.2 Hz, 1H), 7.659 (d, J = 7.2 Hz, 1H), 7.845 (d, J = 9.0 Hz, 1H), 7.930 (d, J = 9 Hz, 1H), 8.869 (s, 1H); ^{13}C NMR δ ($CDCl_3$) 14.189, 64.457, 115.072, 116.464, 124.481, 126.140, 126.933, 128.942, 129.273, 136.860, 138.849, 149.300, 162.205; IR ν (KBr) 2116, 1748; HRMS (EI) calcd for $C_{13}H_{11}N_5O_2$ (M^+) *m/e* 269.0913, found *m/e* 269.0905.

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Supporting Information Available: ^{13}C NMR spectra for the compounds described this paper (26 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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