Reagents for directed modification of biopolymers
9.* Derivatives of 6-azidohexafluoro-2-naphthoic acid. Synthesis and photochemical properties

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The synthesis of new heterobifunctional reagents that are photo-activated, namely, 6-azido-1,3,4,5,7,8-hexafluoro-2-naphthoic acid (**2**), succinimido-6-azido-1,3,4,5,7,8-hexafluoro-2-naphthoate (**3**), and *N*-(*n*-butyl)-1,3,4,5,7,8-hexafluoro-2-naphthamide (**4**), which simulates the product of addition of acid **2** to biopolymers, is described. The photolysis of azides **2** and **4** in methanol and pyridine was studied. Azide **4** is superior to photoreagents based on perfluoroaromatic azides of the benzene series in spectral photosensitivity and rate of photolysis in the UV region (334—405 nm). When azide **4** is irradiated with light at wavelengths above 400 nm, the rate of its photolysis in methanol increases *ca.* 10-fold in the presence of a singlet sensitizer, 9-aminoacridine, which makes reagent **3** promising for designing binary systems sensitive to visible light.

Key words: perfluoroaromatic azides, sensitization, photolysis.

Heterobifunctional photoreagents based on perfluoroaromatic azides of the benzene series are widely used for affine photomodification of proteins[2,3] and complementary-addressed modification of nucleic acids.[4—9] Their advantages include high photoactivity, possibility of using ^{19}F NMR spectroscopy for the study of the photolysis products,[11] high reactivity of species resulting from their photolysis[12,13] due to the electron-accepting properties of F atoms, and hence, enhanced efficiency of biopolymer photomodification.[5—10] However, the electronic spectra of these reagents have absorption maxima in the same wavelength range as biopolymers, which limits their use in complex biological systems. Therefore, development of new reagents with such spectral properties that would enable the photomodification of proteins and nucleic acids in the visible spectral range is a current problem.

The electronic absorption spectra of octafluoronaphthalene derivatives contain a long-wave maximum in the region around 320 nm,[14] while the spectra of naphthyl azides display low-intensity $n\pi^*$ absorption of the azido group in the region of 430—450 nm.[15] In addition, the quantum yields of photodissociation of naphthyl azides are higher than those of phenyl azides and approach unity.[16] It may therefore be assumed that the use of the perfluoronaphthalene system for designing new heterobifunctional photoreagents can be very promising.

In the present work, we describe the synthesis and the photochemical properties of 6-azido-1,3,4,5,7,8-hexafluoro-2-naphthoic acid (**2**) and its ester with *N*-hydroxysuccinimide (**3**) suitable for introducing the residue of acid **2** in proteins and nucleic acid derivatives by a reaction with primary amino groups. In addition, we describe *N*-(*n*-butyl)-1,3,4,5,7,8-hexafluoro-2-naphthamide (**4**), which simulates a part of the addition product of photoreagent **3** to the biopolymers mentioned above. Acid **2** was obtained by the reaction of heptafluoro-2-naphthoic acid (**2**) with sodium azide in DMF. Reaction of acid **2** with *N*-hydroxysuccinimide in the presence of *N*,*N*'-dicyclohexylcarbodiimide (DCC) gave ester **3**. Amide **4** was obtained by the reaction of ester **3** with *n*-butylamine in dioxane (Scheme 1).

It is known that nucleophilic substitution in the perfluoronaphthalene system occurs more readily than in the perfluorobenzene system and that the presence of an electron-accepting substituent at position 2 and the use of an aprotic solvent (DMF) results in a considerable increase in the rate constants of reactions of this type.[17] This made it possible to obtain azide **2** under mild conditions. The optimum molar ratio of the reagents, **1** : NaN_3, is 1 : 1.25 as selected using analytical HPLC. Compound **2** was isolated in 28—37% yields by

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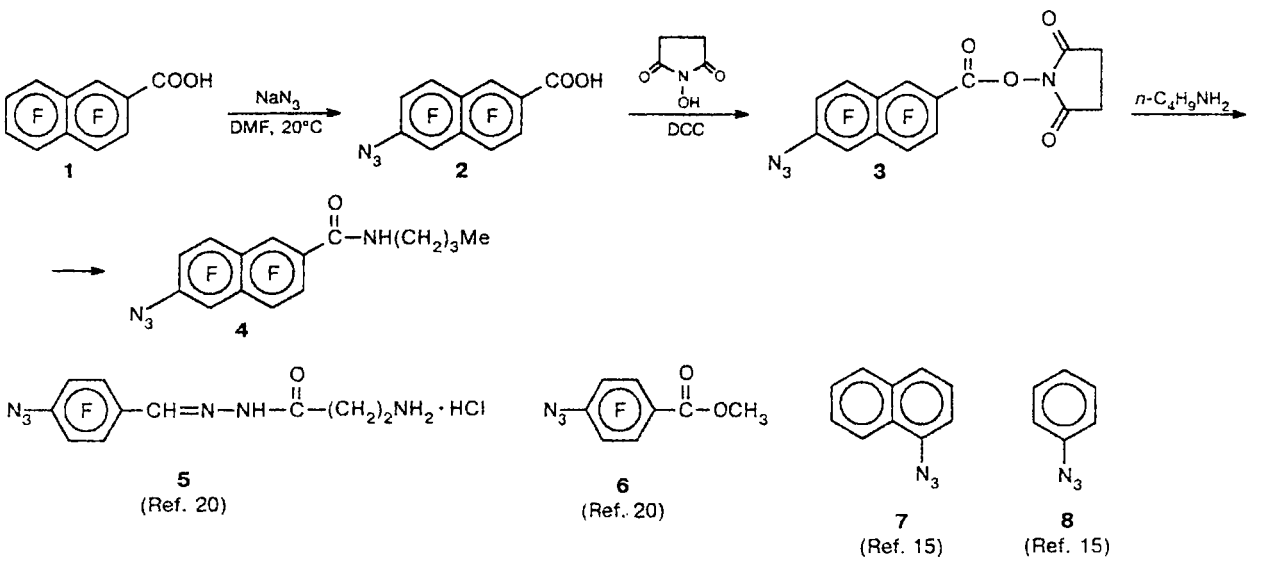
Scheme 1



1 **2** **3** **4**

5 (Ref. 20) **6** (Ref. 20) **7** (Ref. 15) **8** (Ref. 15)

crystallization of the reaction mixture, which contained (according to HPLC data) 75% of a monosubstitution product, 7% of the starting acid **1**, and, presumably, an isomeric monoazide and a product of disubstitution in the perfluoronaphthalene system.

The structure of azide **2** was confirmed by analyzing the ^{19}F NMR spectrum, which contains six signals of equal intensity. Four of them display coupling typical of *peri*-F atoms: for the signals at δ 20.5 and 49.2, $^4J_{FF}$ = 69 Hz, and for those at δ 17.4 and 33.4, $^4J_{FF}$ = 63 Hz. Comparison of these parameters with those for acid **1** (Ref. 18) makes it possible to assign the above signals to the F(8), F(1), F(4), and F(5) atoms, respectively. The signals for the F(4) and F(8) atoms also display pseudotriplet couplings, $J_{FF} \approx 18$ and 16 Hz, indicating that the *ortho*- and *para*-positions relative to them are also occupied by fluorine atoms.[18] The signals for the F(1) and F(5) atoms are doublets with the above-mentioned coupling constants; hence, there are substituents other than F atoms at the *ortho*-positions relative to the F(1) and F(5) atoms, *i.e.*, at positions 2 and 6. In addition, there are signals at δ 20.0 and 27.9 assigned (based on the data from Ref. 18) to the F(7) and F(3) atoms; each of these signals has a doublet splitting with $^3J_{FF}$ (*ortho*-) ≈16 and 18 Hz, respectively, a doublet splitting with $^4J_{FF}$ (*meta*-) ≈ 7 Hz, and a pseudotriplet splitting with $J_{FF} \approx 4$ Hz due to the coupling with two F atoms in the other ring. The signals for F(5) and F(7) are shifted downfield relative to their positions in the spectrum of acid **1** by ~17 and ~11 ppm, respectively, which is caused by replacement of the F atom at the adjacent position by an azido group. Thus, the orientation of nucleophilic substitution of fluorine in compound **1** agrees with the known rules.[19]

After replacement of F(6) by an azido group, the perfluoronaphthalene moiety still contains active centers susceptible to nucleophilic substitution, which has to be taken into account when using reagent **3**. This allows one to introduce functionally valuable substituents into the photoreagent when required.

In a separate experiment, using HPLC monitoring, we studied the stability of the model amide **4** in the presence of *n*-butylamine at room temperature in a solvent (DMSO) that ensured the maximum rate of nucleophilic substitution.[17] It was found that it is only at relatively high concentrations of the nucleophile (0.1—0.2 mol L^{-1}, ~15-fold excess relative to the substrate) that further substitution in the perfluoronaphthalene moiety can be observed in the first hours of the reaction, whereas at concentrations of *n*-butylamine of 0.01—0.02 mol L^{-1} (~1.5-fold excess), no apparent changes are observed in the reaction mixture even after one day. One should expect that in aqueous solutions, where nucleophilic substitution occurs 2—2.5 orders of magnitude more slowly than in aprotic solvents, reagent **4** would be stable.

The photochemical properties of perfluoroaryl azides of the benzene series have been studied sufficiently well,[4,12,13,23] whereas there are no data on the particular features of the photolysis of azides belonging to the perfluoronaphthalene series. The spectral photosensitivity of azides **2** and **4** was studied by conducting their photolysis in methanol under irradiation in various wavelength regions.

In Table 1, the spectral and photochemical characteristics of compounds **2** and **4** are compared to those reported[20] for *p*-azidotetrafluorobenzaldehyde *N*-(3-aminopropionyl)hydrazone hydrochloride (**5**), the most photoactive compound of the perfluoroaryl azides in the benzene series studied. It follows from the data presented that, compared to the UV spectrum of perfluorophenyl azide **5**, the maxima in the spectra of perfluoronaphthyl azides **2** and **4** are shifted bathochromically.

Table 1. Spectral characteristics (λ_{max}) and half-times of photolysis ($\tau_{1/2}$) of perfluoroaromatic compounds in methanol under irradiation in various wavelength regions

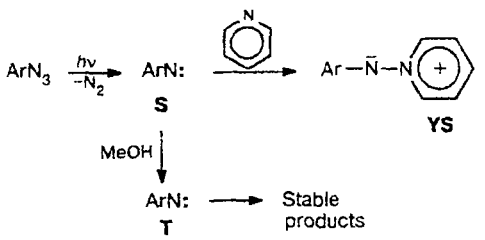
Compound	λ_{max}/nm	$\tau_{1/2}$/s at λ/nm				
		303—365	303—313	334—365	360—385	395—405
2	343	6	30	25	60	1800
4	343	10	60	60	300	9000
5	306	15	80	420	3000	>20000

This causes an increase in their photoactivity compared to that of azide **5**, which is particularly noticeable in the region of 360—385 nm, where the photolysis half-time ($\tau_{1/2}$) for compounds **2** and **4** is smaller than that for azide **5** 50- and 10-fold, respectively. Hence, the spectral photosensitivity and photoactivity of azides of the perfluoronaphthalene series exceed considerably those of the monocyclic analogs, particularly in the long-wave spectral region.

To predict the suitability of perfluoronaphthyl azides for the photomodification of biopolymers, it is necessary to have information on the reactivity, type, and multiplicity of intermediate species generated upon their photolysis. It is known that the photolysis of perfluoroaryl azides (Scheme 2) initially produces a singlet nitrene (**S**), whose reactivity toward nucleophiles can be easily estimated by the chemical traps method.[12,13] A convenient chemical trap for singlet nitrene is pyridine, which forms a stable adduct with nitrene **S**, a singlet nitrene ylide (**YS**), which has characteristic absorption in the visible spectral region (400—430 nm). A simplified modification of the pyridine—ylide method[13] has been used previously[20,21] for comparison of the reactivity in a series of perfluorophenyl azides; the results of this comparison correlate sufficiently well with the data on the efficiency of complementary-addressed photomodification of single-stranded DNA fragments by oligonucleotide derivatives bearing these perfluorophenyl azide residues.[21]

The photolysis of amide **4** in pyridine gives a product with an absorption maximum at 427 nm, *i.e.*, in the region characteristic of ylides **YS**. It was found by HPLC that the yield of ylide **YS** from azide **4** is ~30% as calculated from the results of photolysis in pure pyridine. It can be assumed that, unlike for perfluoroaryl azides of the benzene series, the photolysis of azide **4** does not stop with the formation of nitrene **S** but produces other reactive species, *e.g.*, triplet nitrene **T** (Scheme 2). This agrees with the previously reported data[20,21] that an increase in the chromophore part of perfluoroaryl azides facilitates intercombination conversion of the resulting singlet nitrene into the ground triplet state.

Scheme 2



Methanol can form hydrogen bonds with nitrenes **S** and **T**, which to a different extent stabilizes these intermediates and accelerates intercombination conversion (**S** → **T**)[21] (see Scheme 2). This usually decreases the yield of ylide **YS** and increases the yield of stable products of triplet nitrene transformation. The photolysis of compounds **4**—**6** was studied in a methanol—pyridine mixture (Fig. 1). The results presented suggest that methanol-dependent intercombination conversion of a singlet nitrene into triplet nitrene in perfluoronaphthyl azide **4** (straight line *2*) occurs ~50-fold more easily than that in monocyclic analogs **5** and **6**, the latter being the closest hydrophobic analog of azide **4** among those studied to date[20] (straight line *1*). At concentrations of the protic solvent above 1 mol L^{-1}, the triplet nitrene is the predominating species. The photomodification of biopolymers with azide **4** will most probably result in products of reactions of the triplet nitrene. Since the reactivity of the triplet nitrene is lower than that of the singlet one, the advantages of these reagents are most easily seen when they are used for selective modification of proteins in protein-nucleic acid complexes.

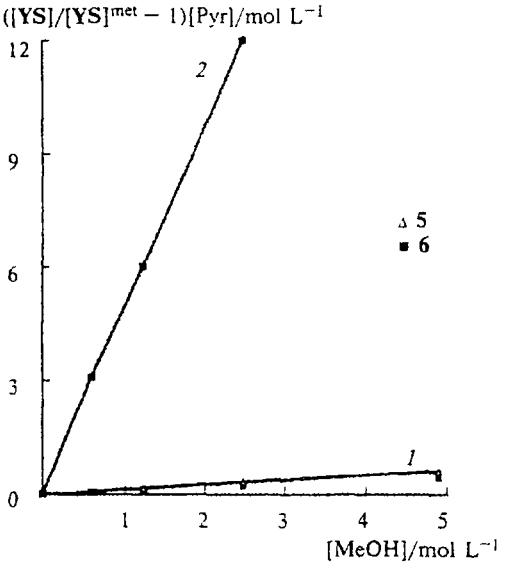


Fig. 1. Dependence of $([YS]/[YS]^{met} - 1) \cdot [Pyr]$ in the photolysis of perfluoroaromatic azides (straight line *1*) and azide **4** (straight line *2*) in a 9.3 *M* solution of pyridine in heptane on the concentration of methanol. [YS] and $[YS]^{met}$ are concentrations of the ylide formed in the absence and in the presence of methanol.

As noted above, an increase in the chromophore part in perfluoroaryl azides facilitates intercombination conversion leading to the formation of a less reactive intermediate **T**. Therefore, to further shift the spectral photosensitivity of photoreagents towards the visible range, we used a more promising approach, namely, the singlet-singlet sensitization based on non-radiating energy transfer from a donor (fluorophore) to an acceptor (azide). The criterion of efficiency of such a process is quenching of donor fluorescence in the presence of an acceptor,[22] which is characterized by a bimolecular quenching constant (k_q).

The quenching of fluorescence was studied and the k_q values were determined using a procedure described previously.[15,22] Table 2 compares data on quenching of fluorescence of singlet sensitizers by the known azides, both perfluoroaromatic **6** and aromatic **7** and **8**, with the data for compound **4** synthesized in this work.

It is evident that in all cases, the k_q values for reagent **4** exceed those for the perfluoroaryl azide **6**, which belongs to the benzene series. This agrees with the literature data on the quenching of fluorescence of singlet sensitizers by non-fluorinated aromatic azides of the benzene and naphthalene series.[15,21] It should be noted that the k_q constants are higher for perfluoroaryl azides **6** and **4** than for their non-fluorinated analogs **8** and **7**, respectively.

The increased tendency of perfluoronaphthyl azides to react with photo-excited states of fluorophores is confirmed by data on direct and sensitized photolysis of azide **4** in the presence of 9-aminoacridine on irradiation with visible light (Fig. 2). One can see that the initial rate of sensitized photolysis of azide **4** (curves *1*—*4*) is ~10 times higher than the rate of direct photolysis (curves *7*—*9*). This effect is consistent with the results obtained previously for compound **5** under similar conditions, where the initial rate of sensitized photolysis of perfluoronaphthyl azide **4** is three times higher than that of perfluorophenyl azide **5**, the most photoactive compound among perfluorophenyl azides.[21] These data indicate that the use of azide **4** is promising for the design of binary systems of photoreagents sensitive to visible light.

By now, photoactive oligonucleotide derivatives have already been obtained using reagent **3**. These derivatives find use for direct and sensitized complementary-addressed modification of nucleic acids.

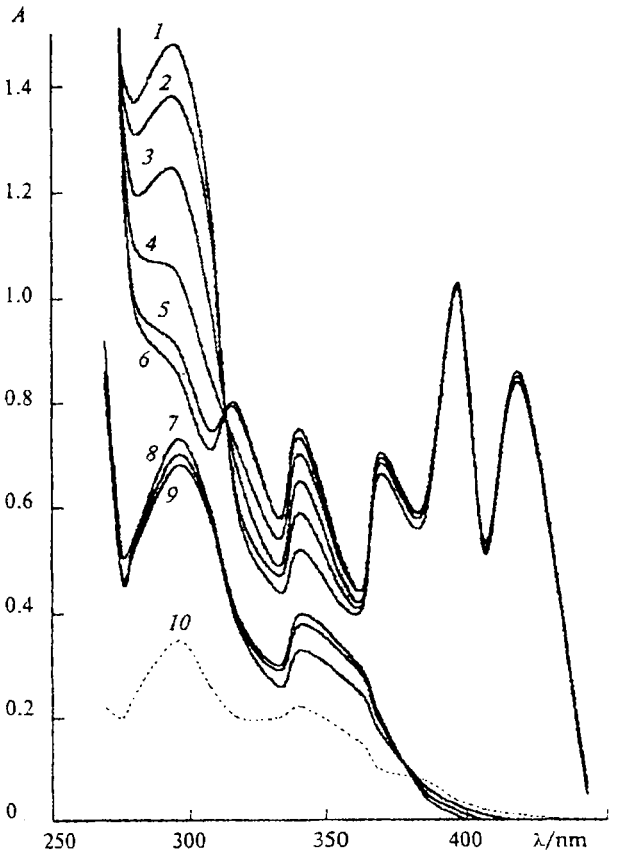


Fig. 2. Changes in the UV absorption spectra in the direct photolysis of azide **4** (curves *7*—*10*) and that sensitized by 9-aminoacridine (curves *1*—*6*) under illumination with visible light > 400 nm in methanol; the concentrations of 9-aminoacridine and azide are 10^{-4} mol L^{-1}. Time of illumination (min): 0 (*1*, *7*); 5 (*2*); 15 (*3*); 30 (*4*, *8*); 60 (*5*, *9*); 600 (*6*, *10*).

Table 2. Excitation (λ_{ex}) and emission (λ_{em}) wavelengths, fluorescence lifetimes (τ_0), and bimolecular constants of quenching (k_q) of the fluorescence of singlet sensitizers by aromatic and perfluoroaromatic azides in methanol

Sensitizer	λ_{ex} / nm	λ_{em} / nm	τ_0 /ns	$k_q \cdot 10^9$/mol L^{-1} s^{-1}			
				8	**7**	**6**	**4**
9-Methylbenzo[1,2]-anthracen-10-yl acetic acid	362	410	24[a]	—	—	2.5	16
Perylene	433	465	5[b]	1.7[b]	9.2[b]	3.3	26
9-Aminoacridine	424	454	7[c]	—	—	2.4	13

Note. The values of λ_{ex} and λ_{em} were measured to within ±5 nm.
[a] Data for 9-methylbenzo[1,2]anthracene; *cf.* Ref. 24.
[b] Ref. 15 [c] Ref. 25.

Experimental

The following reagents were used: sodium azide (Serva), DCC, *N*-hydroxysuccinimide (Sigma), and butylamine ("pure" grade). Organic solvents were dried and purified using standard procedures. Analytical HPLC was performed on a Milikhrom-4 microcolumn chromatograph (Russia), using a 2×64 mm column, Lichrosorb RP-8 sorbent, linear gradient of methanol (50—100%) in 0.05 *M* Tris-HCl buffer solution (pH 7.5) containing 0.05 *M* $LiClO_4$ as the solvent, and UV detection (λ 250, 280, and 320 nm). TLC was performed on Silufol UV-254 (Kavalier, Czechoslovakia) in the following systems: benzene—methanol—acetone—acetic acid, 14 : 4 : 1 : 1 (v/v) (**A**) and chloroform (**B**).

NMR spectra were recorded on a Bruker WP-200-SY spectrometer using C_6F_6 as the internal standard. Mass spectra were obtained on a Finnigan MAT 8200 spectrometer (EI, 70 eV, direct inlet of the sample). UV spectra were obtained on a Specord-M40 instrument. Fluorescence spectra of solutions in methanol were recorded on a Hitachi MPF-4 spectrofluorimeter. IR spectra were recorded in KBr pellets on a UR-20 instrument. Melting points were measured on a Koffler heating stage. The photochemical properties of the reagents were studied by irradiating their solutions with non-condensed light from a DRK-120 high-pressure mercury lamp from an OI-18A illuminator (LOMO) passed through the following sets of light filters (λ/nm; W/mW cm^{-2}): ZhS-3, UFS-2 (303—313; 1), BS-12, UFS-1 (303—365; 3.5), BS-6, UFS-6 (334—365; 3), FS-1, UFS-1 (365—390; 2), BS-8, PS-13 (395—405; 1.5), ZhS-10 (> 400). The power of irradiation (W) at various wavelengths was determined by means of a ferrioxalate actinometer.[26]

Photolysis in methanol was performed at a 0.15 mM concentration of azides. The photoreagents were irradiated until changes in the UV spectra ceased or until the isosbestic points changed abruptly.

Photolysis in pyridine was performed at a 0.18 mM concentration of azides using FS-1 and UFS-1 light filters. The optimum time of irradiation for compounds **2** and **4** was 10 min. Photolysis of compounds **4**—**6** in pyridine solutions with an admixture of methanol was performed at a 9.3 mol L^{-1} concentration of pyridine. Heptane was used as the inert solvent. The concentrations of methanol were 0, 0.6, 1.23, 2.48, and 4.9 mol L^{-1}. Syntheses of azides and reactions of the latter were carried out in dark-glass vessels in a place protected from light.

Heptafluoro-2-naphthoic acid (1) was obtained by a known procedure[27] from a mixture of 1- and 2-chloroheptafluoronaphthalenes and purified by recrystallization from benzene. Its properties agreed with the literature data.

6-Azido-1,3,4,5,7,8-hexafluoro-2-naphthoic acid (2). NaN_3 (0.273 g, 4.195 mmol) was added to a solution of compound **1** (1 g, 3.356 mmol) in DMF (6.5 mL), and the reaction mixture was stirred at ~20 °C. The reaction was monitored by HPLC. After 45 h, the reaction mixture was diluted with 0.5% aqueous $NaHCO_3$ (30 mL; pH 8), and the precipitate was separated by centrifugation. The supernatant was acidified with 6 mL of concentrated HCl (pH < 1) and kept for 0.5 h at 0 °C, and the precipitate that formed was filtered off and washed with water (2×2 mL). The product (0.7 g) was recrystallized from 90% aqueous methanol, washed with 50% aqueous methanol, and dried *in vacuo* to give 0.3 g (28.0%) of compound **2**. R_f 0.56 (**A**), m.p. 171—173 °C (decomp.). Found (%): C, 41.52; H, 0.25; N, 12.46. $C_{11}HF_6N_3O_2$. Calculated (%): C, 41.14; H, 0.31; N, 13.08. ^{19}F NMR (acetone-d_6), δ: 49.21 (ddd, F(1)); 33.47 (ddd, F(5)); 27.94 (ddt, F(3)); 20.52 (dtd, F(8)); 19.96 (ddt, F(7)); 17.38 (dt, F(4)). $J_{1,8} = J_{8,1} = 69$ Hz, $J_{4,5} = J_{5,4} = 63$ Hz, $J_{4,3} = J_{3,4} = J_{4,1} = J_{1,4} = 18$ Hz, $J_{7,8} = J_{8,7} = J_{8,5} = J_{5,8} = 16$ Hz, $J_{5,7} = J_{7,5} = J_{1,3} = J_{3,1} = 7$ Hz, $J_{1,5} = J_{5,1} \sim J_{3,7} = J_{7,3} \sim J_{3,8} = J_{8,3} \sim J_{4,7} = J_{7,4} \sim 4$ Hz, $J_{1,7} = J_{7,1} \sim J_{3,5} = J_{5,3} \sim 2$ Hz, $J_{4,8} = J_{8,4} \sim 1$ Hz. UV (MeOH), λ_{max}/nm (ε): 217 (31500), 255 (40700), 292 (8900), 343 (4300). IR, ν/cm^{-1}: 2140 (—N_3), 1720 (C=O), 1660, 1480 (C=C arom.), 1140, 980 (C—F). MS, m/z (I_{rel} (%)): 321 [M]$^+$ (55), 293 [M—N_2]$^+$ (100), 276 [M—N_2—OH]$^+$ (43), 248 [M—N_2—COOH]$^+$ (58), 230 [M—91]$^+$ (85). Found M$^+$: 320.99732. $C_{11}HF_6N_3O_2$. Calculated M$^+$: 320.99729.

Succinimido-6-azido-1,3,4,5,7,8-hexafluoro-2-naphthoate (3). A solution of DCC (0.138 g, 0.668 mmol) in 1 mL of dioxane was added to a solution of acid **2** (0.200 g, 0.625 mmol) and *N*-hydroxysuccinimide (0.075 g, 0.656 mmol) in 2 mL of dry dioxane, and the mixture was stirred for 3 h at ~20 °C. The precipitate of dicyclohexylurea was separated by centrifugation and washed with dioxane. The supernatant was concentrated, and the residue was recrystallized from chloroform, washed with a chloroform—hexane mixture (1 : 1, v/v), and dried *in vacuo* to give 0.198 g (76%) of compound **3**, m.p. 164—166 °C (decomp.), R_f 0.33 (**B**). ^{1}H NMR ($CDCl_3$), δ: 2.92 (s, —CH_2—CH_2—). ^{19}F NMR ($CDCl_3$), δ: 52.50 (dm, F(1)); 29.78 (dm, F(5)); 27.33 (dm, F(3)); 19.51 (dm, F(8)); 17.59 (m, F(7)); 15.30 (dm, F(4)). IR, ν/cm^{-1}: 1750, 1780 (C=O), 2140, 2150 sh. (—N_3). MS, m/z (I_{rel} (%)): 418 [M]$^+$ (21), 390 [M—N_2]$^+$ (15), 304 [M—R]$^+$ (64), 276 [M—N_2—R]$^+$ (100), 248 [M—N_2—COR]$^+$ (100) (R = succinimidooxy). Found: M$^+$ 418.01367, $C_{15}H_4F_6N_4O_4$. Calculated: M$^+$ 418.01366.

***N*-(*n*-Butyl)-6-azido-1,3,4,5,7,8-hexafluoro-2-naphthamide (4).** *N*-Hydroxysuccinimide (0.038 g, 0.327 mmol) was added to a solution of acid **2** (0.1 g, 0.311 mmol) in dry dioxane (1.5 mL), and a solution of DCC (0.069 g, 0.333 mmol) in dry dioxane (0.5 mL) was then added. The reaction mixture was stirred for 3 h at ~20 °C. The precipitate of dicyclohexylurea was separated by centrifugation. *n*-Butylamine (0.046 mL, 0.467 mmol) was added to the supernatant. After 10 min, the reaction mixture was centrifuged, and the supernatant was concentrated. The precipitate was dissolved in chloroform and washed with water (2×1 mL). The chloroform solution was dried with $MgSO_4$ and concentrated, and the residue was recrystallized from chloroform. The resulting light crystals were dried *in vacuo* to give 0.088 g (75%) of compound **4**, m.p. 142—144 °C, R_f 0.45 (**B**). UV (MeOH), λ/nm (ε): 217 (23500), 255 (39000), 292 (8000), 320 (3300), 343 (4100). IR, ν/cm^{-1}: 3290, 3340 (N—H); 1640, 1670 (C=O); 2150 (—N_3). MS, m/z (I_{rel} (%)): 376 [M]$^+$ (26), 348 [M—N_2]$^+$ (28), 306 [M—N_2—CH_2=$CHCH_3$]$^+$ (100), 276 [M—N_2—$NH(CH_2)_3CH_3$]$^+$ (89), 248 [M—N_2—$CONH(CH_2)_3CH_3$]$^+$ (80). Found: M$^+$ 376.07591. $C_{15}H_{10}F_6N_4O$. Calculated: M$^+$ 376.07587.

Fluorescence quenching. A methanolic solution of a sensitizer (10 μM) was excited at λ_{ex}, and the fluorescence intensity was recorded at λ_{em} as a function of the concentration of perfluoroaryl azides **4** and **6**: 0, 1, 2, 5, 10, 20, 50, 100, and 200 mM. The bimolecular quenching constant was determined using the equation

$$F_0/F = 1 + \tau_0 \cdot k_q \cdot [Q],$$

where F_0 and F are the intensities of fluorescence of the sensitizer in the absence and in the presence of an azide as a quencher (Q), and τ_0 is the life-time of sensitizer fluorescence.

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