Synthesis and fluorescent properties of new derivatives of 4-amino-7-nitrobenzofurazan

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Abstract

The following new compounds were obtained by reacting 4-chloro-7-nitrobenzofurazan (NBD-Cl, **1**) with five primary amines: **3b** with a benzo-crown ether 18C6; **3c** with an N-(α-naphthyl)-ethylenediamine group; **3d**, with a 2,2,6,6-tetramethylpiperidin-N-oxyl group; **3e**, with an α-picolyl group; and **3f**, derived from tris(hydroxymethyl)aminomethanol. Also, from the reaction of **1** with N-methylhydroxylamine an N-hydroxy-N-methyl-NBD derivative (**3g**) was prepared. All these six new NBD derivatives **3b-g** were studied (in comparison with the known compound **3a** prepared from **1** and aniline) for their physical and chemical properties, with special emphasis on hydrophobicity, UV-Vis, fluorescence, using also structural studies trough QSPR.

Keywords: 4-Amino-7-nitrobenzoxadiazole derivatives, UV-Vis, fluorescence, EPR, hydrophobicity, QSPR

Introduction

Many 4-substituted-7-nitro-2,1,3-benzoxadiazoles (NBD derivatives) have a strong fluorescence which has led to their use in bioanalytical chemistry.[1-23] Their benzoxadiazole ring system also been called 3,4-benzo-1,2,5-oxadiazole or benzofurazan. The usual synthesis is based on the

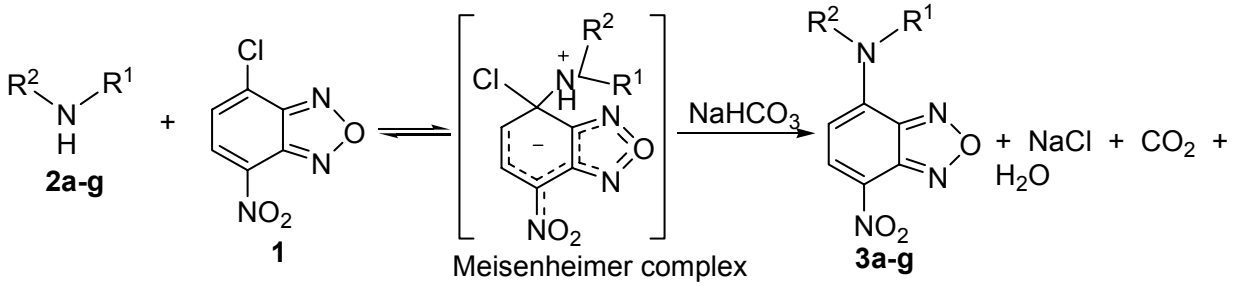
nucleophilic substitution of halogens from 4-halo-7-nitrobenzofurazan, with the halogen being either chlorine (NBD-Cl) or fluorine.[1-23] Some of these compounds have biological activity as antileukemic, immunosuppressive, or monoamine oxidase inhibiting activity.[1,2,24]

Previous papers from our laboratories have reported studies of NBD derivatives having 4-aryloxy, 4-formylaryloxy groups or with amino acids.[25-27] In the present article we describe the synthesis of six new NBD derivatives (**3b**–**3g**) prepared from NBD-Cl (**1**) and five primary amines (**2b**–**2f**) or N-methylhydroxylamine (**2g**). Their properties are described and compared with the known compound **3a**[1,2,10,12,15,16] obtained from **1** and aniline (**2a**). Spectral characterization was performed by ^{1}H- and ^{13}C-NMR spectrometry, IR and UV-Vis absorption spectroscopy, electron spin resonance (EPR for **3d**), and the hydrophobicity was measured by reverse phase thin-layer chromatography (RP-TLC).

Results and Discussion

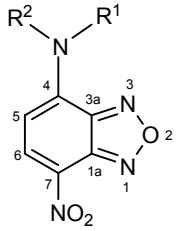
Synthesis of compounds 3a–3g

Starting from **1** and primary amines such as aniline (**2a**), amino-benzo-crown[18C6] (**2b**), *N*-(α-naphthyl)-ethylenediamine dihydrochloride (**2c**), 4-amino-2,2,6,6-tetramethylpiperidin-*N*-oxyl (4-amino-TEMPO, **2d**), 2-(methylamino)pyridine (**2e**), tris(hydroxymethyl)aminomethane (**2f**) or from N-methylhydroxylamine hydrochloride (**2g**), the NBD derivatives **3a**–**3g** displayed in Table 1 were prepared. The reaction was carried out in a convenient solvent (methanol, ethanol, acetonitrile), under heating. For **3b**–**3e** and **3g** the addition of sodium hydrogen carbonate was needed. The appearance of a red-brown color and theoretical studies prove the intermediacy of Meisenheimer complex (Scheme 1), according to the literature data;[24-26,28,29] in our case, the red-brown colored reaction medium, by treating with acid, turned to yellow-orange, thus proving the conversion of the Meisenheimer complex into the reaction product which could be isolated. The crystalline **3a** was obtained directly, but the other compounds needed purification by preparative TLC.

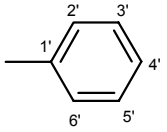
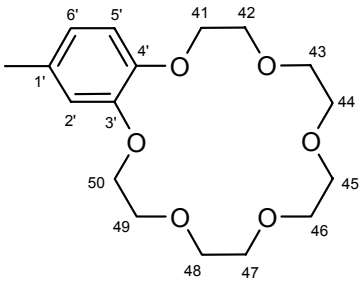
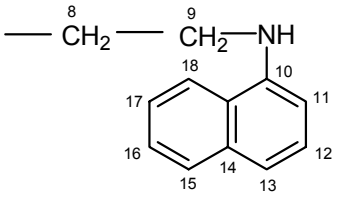
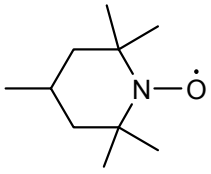
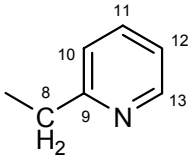
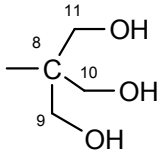


Scheme 1. Synthesis of compounds **3a – 3g.**

Table 1. Structures of the NBD derivatives **3a** – **g**



3a-g

Compound	R^1	R^2
3a	H	
3b	H	
3c	H	
3d	H	
3e	H	
3f	H	
3g	$^8CH_3$	OH

NMR Spectra of compounds 3a–g

The NMR data of compounds **3a-c, 3e-g** (Table 2) confirm the proposed structure.

Table 2. ^{1}H-NMR and ^{13}C- NMR data of compounds **3a-g** (δ ppm; *J* Hz)

Compound	NMR-spectra
3a	^{1}H-NMR ($CDCl_3$, δ ppm, *J* Hz): 8.46(d, 1H, H-5, 8.6); 7.9-8.0(br, 1H, NH, deuterable); 7.54(dd, 2H, H-3'-5', 7.8, 8.5); 7.44(dd, 2H, H-2'-6', 1.3, 8.5); 7.38(tt, 1H, H-4', 1.3, 7.8); 6.75(d, 1H, H-6, 8.6). ^{13}C-NMR($CDCl_3$, δ ppm): 144.73(C-4); 143.84(C-3a); 141.09(C-1'); 136.60(C-7); 125.57(C-1a); 136.03(C-5); 130.13(C-3'-5'); 127.28(C-4') 123.59(C-2'-6'); 100.93(C-6).
3b	^{1}H-NMR ($CDCl_3$, δ ppm, *J* Hz): 8.41(d, 1H, H-5, 8.7); 8.20-8.30(br, 1H, NH, deuterable); 6.95-6.90(m, 3H, H-2'-5'-6'); 6.55(d, 1H, H-6, 8.7); 4.18(m, 4H, H-41-50); 3.95(m, 4H, CH_2); 3.78(m, 4H, CH_2); 3.73(m, 4H, CH_2); 3.70(s, 4H, H-45-46). ^{13}C-NMR($CDCl_3$, δ ppm): 149.57(C-3'); 147.96(C-4'); 144.54(C-4); 143.93(C-3a); 142.15(C-7); 129.62(C-1'); 124.79(C-1a); 136.27(C-5); 117.05(C-6'); 113.69(C-5'); 109.92(C-2'); 100.75(C-6); 71.04(CH_2); 70.62(CH_2); 70.51(CH_2); 70.44(CH_2); 70.27(CH_2); 69.43(CH_2); 69.26(CH_2); 69.20(CH_2); 69.03(CH_2); 68.82(CH_2).
3c	^{1}H-NMR (dmso-d_6, δ ppm, *J* Hz): 9.50(br, 1H, NH, deuterable); 8.42(d, 1H, H-5, 8.9); 8.06(dd, 1H, H-18, 1.0, 7.1); 7.74(dd, 1H, H-15, 1.2, 6.4); 7.40(m, 2H, H-16-17); 7.29(t, 1H, H-12, 7.7); 7.11(d, 1H, H-13, 7.7); 6.62(1H, H-11, 7.7); 6.41(d, H-6, 8.9); 3.80(br, 2H, H-9); 3.60(t, 2H, H-8, 5.8). ^{1}H-NMR ($CDCl_3$, δ ppm, *J* Hz): 8.39(d, 1H, H-5, 8.6); 7.84(m, 2H, H-13-15); 7.52÷7.35(m, 5H, H-11-12-16-17-18); 6.19(d, 1H, H-6, 8.6); 6.86(bs, 1H, H-8', deuterable); 6.70(bs, 1H, H-9', deuterable); 3.92(bs, 2H, H-9); 3.82(t, 2H, H-8, 6.2). ^{13}C-NMR (dmso-d_6, δ ppm): 145.32(Cq-10); 144.38(Cq-4); 143.47(Cq-3a); 137.92(Cq-7); 134.08(Cq-14); 123.07(Cq-1a); 120.86(Cq-19);137.81(C-5); 99.23(C-6); 128.03(C-15); 126.78(C-12); 125.70(C-17); 124.08(C-18); 121.44(C-16); 115.91(C-13); 103.13(C-11);42.25(C-8 or C-9); 41.41(C-9 or C-8).
3c in TFA	^{1}H-NMR ($CDCl_3$+TFA, δ ppm, *J* Hz): 8.39(d, 1H, H-5, 8.7); 8.02(dd, 1H, H-11, 8.4, 0.9); 7.98(m, 2H, H-18-16); 7.70(dd, 1H, H-13, 0.9, 7.6); 7.65(m, 2H, H-15-17); 7.52(dd, 1H, H-12, 7.7, 8.8); 6.26(d, 1H, H-6, 8.7); 4.18(s, 4H, H-8 and H-9). ^{13}C-NMR($CDCl_3$+TFA, δ ppm): 144.13(Cq-10); 143.83(Cq-4); 143.43(Cq-3a); 134.69(Cq-7); 134.68(Cq-14); 124.93(Cq-1a); 124.33(Cq-19); 136.81(CH-5); 131.75(CH); 129.78(CH); 129.08(CH); 128.01(CH); 125.24(CH); 121.44(CH); 118.55(CH); 100.62(CH-6); 51.08(C-9); 40.00(C-8).
3e	^{1}H-NMR (dmso-d_6, δ ppm, *J* Hz): 9.9(br, 1H, HN); 8.53(dd, 1H, H-13, 1.0, 4.6); 8.5(d, 1H, H-5, 8.9); 7.78(td, 1H, H-11, 7.8, 1.0); 7.42(d, 1H, H-10, 7.8); 7.31(dd, 1H, H-12, 4.6, 7.8); 6.33(d, 1H, H-6, 8.9); 4.80(br, 2H, H-8). ^{13}C-NMR (dmso-d_6, δ ppm): 156.26(C-9); 149.39(C-13); 145.17(C-4); 144.12(C-3a);

	138.61(C-7); 137.97(C-5); 137.36(C-11); 122.98(C-10); 121.80(C-12); 125.97(C-1a); 100.09(C-6); 48.31(C-8).
3f	^{1}H-NMR (dmso-d_6, δ ppm, *J* Hz): 8.51(d, 1H, H-5, 9.0); 7.54(br, 1H, NH, deuterable); 6.86(d, 1H, H-6, 9.0); 5.13(t, 3H, HO, deuterable, 5.4); 3.77(d, 6H, H-9-10-11, 5.4). ^{13}C-NMR(dmso-d_6, δ ppm): 145.39(C-4); 144.52(C-3a); 143.88(C-7); 137.93(C-5); 121.13(C-1a); 102.30(C-6); 64.73(C-9-10-11); 64.13(C-8).
3g	^{1}H-NMR (dmso-d6, δ ppm, *J* Hz): 9.50(br, 1H, OH, deuterable); 8.50(d, 1H, H-5, 8.7); 6.29(d, 1H, H-6, 8.7); 3.05(d, 3H, H-8, 4.0). ^{13}C-NMR(dmso-d_6, δ ppm): 145.71(C-4); 144.23(C-3a); 144.02(C-7); 137.88(C-5); 120.89(C-1a); 98.97(C-6); 30.14(C-8).

Assignments in Table 2 are using the atom numbering indicated in Table 1. No NMR data are reported for the paramagnetic compound **3d**. Compound **3c**, with two amino groups, is converted into an ammonium salt by protonation of the naphthylamino group (the strongly electron-withdrawing NBD group cancels the basicity of the adjacent amino group). The changes in the NMR spectra are evident – there are significant differences for C-9 and protons H-8 and H-9 in the ethylene group, small changes for H-11 in the naphthyl group, and no changes in the NBD moiety.

Hydrophobic/hydrophilic balance of compounds 3a–g

All biological uses of chemical compounds depend on how they interact with biomembranes, and such interactions are governed by the hydrophobic/hydrophilic balance, so that we had to include such effects in the present study. Following previous reports,[25,26] the hydrophobic/hydrophilic balance of compounds **3a–g** was studied experimentally by reverse phase TLC (RP-TLC), a simple, efficient, and precise method. The molecular hydrophobicity R_{M0} was determined by means of equations (1) and (2), using the data presented in Table 3.

$$R_M = \log(1/R_f - 1) \quad (1)$$

$$R_M = R_{M0} + bK \quad (2)$$

The hydrophobicity of compounds **3a-g** decreases in the order **3c > 3a** > **3d** >**3e** > **3g** > **3b** > **3f** (hydrophilicity increasing obviously in the reverse order). The NBD group has log P = 1.69.[35] The remaining R^1R^2N moiety combines its effect leading to increased hydrophobicity due to the presence of phenyl, naphthyl, pyridine and 2,2,6,6-tetramethylpiperidyl (**3a, 3c-e)** or to decreased hydrophobicity in the presence of OH groups and the crown ether macrocycle (**3b, 3f, 3g)**.

Table 3. Experimental hydrophobicity (R_{M0}, b)[a] and calculated (log P)[30] for **3a – g**

Comp.	Exp. R_M in aqueous ethanol, conc.(v/v)				R_{M0}	b	Statistical parameters			Calcd. log P
	80%	70%	60%	50%			R	F	SD	
3a	-0.508	-0.281	0.067	0.407	1.932	-0.031	-0.996	238	0.045	4.39
3b	0.103	0.097	0.216	0.320	0.685	-0.010	-0.939	14	0.145	3.34
3c	-0.447	-0.165	0.301	0.733	2.708	-0.040	-0.995	202	0.063	5.18
3d	-0.574	-0.281	0.067	0.322	1.857	-0.030	-0.998	641	0.020	3.92
3e	-0.508	-0.407	-0.112	0.281	1.543	-0.027	-0.971	32	0.104	2.94
3f	-1.255	-0.985	-0.740	-0.619	0.497	-0.021	-0.987	76	0.055	1.80
3g	-0.727	-0.553	-0.301	-0.084	1.001	-0.022	-0.998	436	0.023	2.68

[a] Silica gel RP-18 F_{254} (Merck); R_{M0} = molecular hydrophobicity (eq. 2); b = change in R_M value caused by increasing the concentration (K) of the organic component in the mobile phase (eq. 1); R = correlation coefficient for parameters R_{M0} and b in eq. 2.[31-34]

On calculating logP values using fragmental constants,[30] a relatively good correlation (R^2=0.857) with experimental data for R_{M0} was obtained for compounds **3a**–**g** (Figure 1).

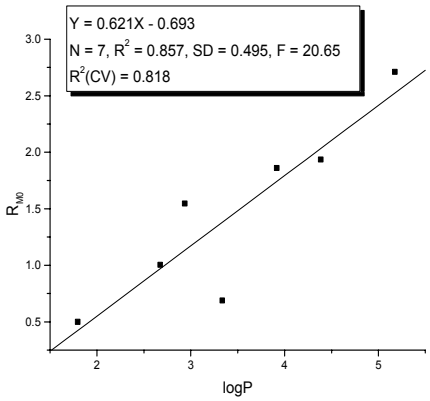


Figure 1. R_{M0} *vs* logP for compounds **3a**–**g.**

Electronic absorption spectra and fluorescence of compounds 3a-g

UV-Vis spectra

Compounds **3a**–**g** are reddish or brown in crystalline state, and their solutions in organic solvents are yellow, orange, or red. All are soluble in absolute ethanol, so that one can make comparisons between their electronic absorption bands. As seen from Table 4, all compounds present a strong band in the visible region (λ_{max} = 457 – 483 nm) due to the NBD chromophore.[3,25] The differences are due to extended conjugation with the acceptor NBD group[10,24,36] for aromatic

susbtituents at the amino group (**3a**, **3b**, which absorb at higher wavelengths), whereas the remaining compounds having alkyl, hydroxy, or aralkyl groups absorb at lower wavelengths.

Calculated Mulliken net atomic charges on the amino nitrogen (NAC_N) using the AM1 algorithm for molecular geometries,[37] and the CODESSA program [38] are presented in Table 5 together with the values found by a simple linear correlation, eq. (3), where NAC_N is the net atomic charge for the nitrogen atom, and SD is the standard deviation (calculated and experimental values had two decimals).

$$\lambda_{max}(\text{calc.}) = -145.9(\pm 25.72)NAC_N + 434.9 \quad (3)$$

$$N = 7;\ R^2 = 0.865;\ SD = 3.648;\ F = 32.2;\ R^2_{\text{cross-valid.}} = 0.815$$

Table 4. UV-Vis spectral data of compounds **3a**–**g** in absolute ethanol

Comp.	Conc.(M)	λ_{max} (nm)	$\varepsilon \times 10^3$ (L × mole^{-1} × cm^{-1})
3a	1.21×10^{-4}	279 (sh) 330 475	2.80 6.28 17.60
3b	1.20×10^{-4}	279 334 483	2.16 2.75 6.75
3c	4.25×10^{-4}	333 465	1.08 1.43
3d	4.25×10^{-4}	331 464	1.08 2.23
3e	4.25×10^{-4}	261 (sh) 326 457	0.611 0.752 1.88
3f	1.43×10^{-4}	265 (sh) 330 463	1.39 3.49 7.83
3g	4.25×10^{-4}	332 462	1.41 2.96

Table 5. Net atomic charges on the amino nitrogen (NAC_N), and λ_{max} (exp. in Table 4 and calc. with eq. 3, in nm) for compounds **3a**–**g** in absolute ethanol

Compound	NAC_N	λ_{max}(exp)[a]	λ_{max}(calc.)	Residual
3a	-0.285	475	476	-0.59
3b	-0.294	483	477	6.18
3c	-0.229	465	468	-3.24
3d	-0.216	464	466	-1.83
3e	-0.126	457	453	3.66
3f	-0.204	463	464	-1.78
3g	-0.185	462	462	0.48

[a] see Table 4

Compound **3b** with the 18C6 is able to form complexes with some alkali cations.[39,40] Indeed, an acetonitrile solution of compound **3b** undergoes a slight hypsochromic shift on treatment with potassium perchlorate (molar ratio1:1) from 480 nm to 477 nm, with an isosbestic point at λ=514 nm.

General characteristics for the fluorescence of compounds 3a–g

It is known that NBD compounds with a 4-alkylamino substituent are fluorescent,[1-23,41] but only weakly fluorescent when they have a 4-arylamino substituent such as phenyl (**3a**).[1,2,10,12,15,16] Among compounds **3a**–**g**, only compounds **3e-g** are strongly fluorescent in solid state and in most solvents. Compounds **3a** and **3d** are weakly fluorescent in solid state and in most solvents. Compound **3b** is not fluorescent either pure or as complex with $KClO_4$. Compound **3c** is not fluorescent in solid state, but is weakly fluorescent in some solvents (e. g. dichloromethane, benzene, and toluene); a more detailed account will be seen below.

By choosing the excitation wavelength at λ_{ex} = 450 nm and absolute ethanol as solvent ($E_T(30)$=51.9),[42] the characteristic data for the fluorescence of compounds are presented in Table 6. One can observe that the emission wavelength (λ_{em} =524-545 nm) agrees with the known range for NBD derivatives.[1-23,41] The λ_{em} values decrease in the order λ_{em} **3a** > λ_{em} **3d** = λ_{em} **3f** > λ_{em} **3e** > λ_{em} **3g**; the quantum yields (Φ) decrease in the order Φ **3e** > Φ **3f** > Φ **3g** > Φ **3d** >>Φ **3a** (the last compound has a very low a value); the natural lifetimes (τ_0) decrease in the order τ_0**3e** > τ_0**3d** > τ_0**3g** > τ_0**3f**; and the calculated lifetimes (τ) according to the Strickler-Berg formula (4)[43] which involves the quantum yield (Φ) decrease in the order: τ **3e** > τ **3g** > τ **3f** > τ **3d**.

$$\frac{1}{\tau_0} = 2.88\times10^{-9} n^2 \frac{\int I_F(\nu_F)d\nu_F}{\int I_F(\nu_F)\nu_F^{-3}d\nu_F} \times \int \frac{\varepsilon(\nu_A)}{\nu_A} d\nu_A \qquad (4)$$

where: τ_0 is the lifetime, ν is the wavenumber of the maximum of the absorption band, n is the refractive index of the solvent (1.3595 for ethanol), I_F is the fluorescence intensity, ε is the molar absorption coefficient, and $\tau = \tau_0 \cdot \Phi$.

In the case of the paramagnetic compound **3d** one must ascribe the quenching of fluorescence to an intermolecular process, similarly to literature data, due to the 4-amino-TEMPO free radical.[44-46]

Table 6. Fluorescence characteristics λ_{em}, quantum yield (Φ), natural lifetime (τ_0), and calculated lifetime (τ) in absolute ethanol for compounds **3a, 3d–g** for λ_{ex} = 450 nm

Compound	λ_{em} (nm)	Φ [a,b]	τ_0 (ns)	τ (ns)
3a	545	Very low [c]		
3d	531	0.0016	79.05	0.13
3e	526	0.0587	104	6.10
3f	531	0.0393	24.5	0.96
3g	524	0.0298	62.6	1.86

[a]conc. (**3a**)=1.21×10^{-4} M, conc. (**3d,3e,3g)**=4.25×10^{-4} M; conc. (**3f**)=1.43×10^{-4} M
[b] compared to the quinine bisulfate (in 0.1N H_2SO_4, Φ=0.55)
[c] 2.03×10^{-5} mol/L

As discussed above, the electronic absorption spectra, for the three compounds **3d,3e,3g** that have a significant fluorescence, it was possible to correlate the fluorescence lifetime τ (which involves also the quantum yield) with the calculated net atomic charge for the amino nitrogen atom (NAC_N) by the equation (5), as seen in Table 7.

$$\tau = 67.32\ (\pm\ 4.791)\ NAC_N + 14.53\ (\pm\ 0.837) \quad (5)$$

$$N = 3 \qquad R^2 = 0.995\ SD = 0.275 \qquad F = 197.4 \quad R^2_{cross\text{-}valid.} = 0.980$$

Table 7. Calculated values of net atomic charge on the amino nitrogen (NAC_N) by CODESSA program and τ (exp. in Table 6 and calc. with eq. 5) for compounds **3e-g**

Compounds	NAC_N	τ(exp.)[a]	τ(calc.)	Resid.
3e	-0.126	6.10	6.05	0.05
3f	-0.204	0.96	0.80	0.16
3g	-0.185	1.86	2.07	-0.21

[a)] See Table 6

Fluorescence of compound 3d

The paramagnetic compound **3d** is weakly fluorescent due to intermolecular quenching. The EPR spectrum has three lines (Figure 2) due to a hyperfine coupling with a_N = 14.79 Gauss (in methylene chloride) in agreement with that of 4-amino-TEMPO.[47]

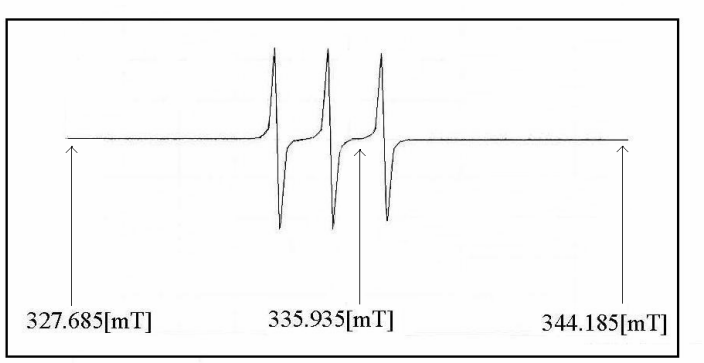
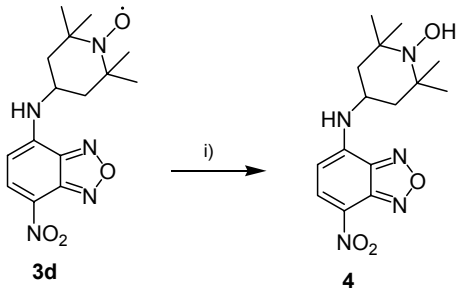


Figure 2. EPR spectrum of **3d** in dichloromethane.

With an excess of ascorbic acid in absolute ethanol, the solution becomes strongly fluorescent in a few minutes (the intensity of the fluorescence increases about six times, as seen in Figure 3), due to the formation of hydroxylamine **4** (Scheme 2). Compound **4** was detected by TLC (R_f **3d** = 0.907, R_f **4** =0.372, on silica gel with methylene chloride:methanol 9.5:0.5 v/v). The process described in Scheme 2 is reversible, because oxidation of **4** (with PbO_2, Ag_2O, $KMnO_4$, even with air) produces **3d**.



Scheme 2. Reduction of **3d** (i = ascorbic acid, molar ratio **3d:** ascorbic acid =1:6).

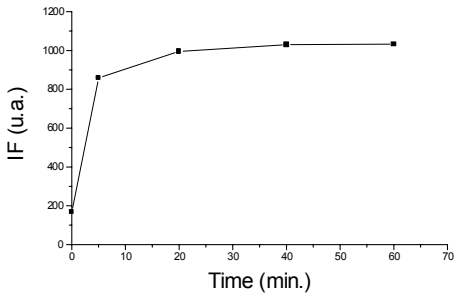


Figure 3. Variation of the fluorescence intensity (I_F) during the reduction of **3d (**in absolute ethanol) with an excess of ascorbic acid.

These fluorescence and paramagnetic properties of compound **3d** may lead to applications as a *molecular probe* for biological redox processes.

Fluorescence of compound 3c

In absolute ethanol, compound **3c** is not fluorescent, but in less polar solvents (benzene, toluene) a weak fluorescence (Table 8) due to the NBD group was detected (λ_{ex}=450 nm, λ_{em}=505 – 512 nm).

In acetic acid which has the same polarity as absolute ethanol, a weak fluorescence has also been observed. However, the fluorescence increases significantly in the presence of strong acids such as trifluoroacetic acid and 4-toluenesulfonic acid (Table 9), when the α-naphthylamino group becomes protonated affording cation **5** (Scheme 3). Trifluoroacetic acid introduces a significant hypsochromic shift (16 nm) in the visible spectrum, and the protonated compound **5** has the highest value for Φ (Table 9).

Table 8. The effect of solvent polarity on the absorption and fluorescence spectra of compound **3c** (using λ_{ex} = 450 nm)

Solvent and $E_T(30)$[42]	Conc. of compound **3c** (M)	λ_{max} (nm)	$\varepsilon \times 10^3$ ($L \times mol^{-1} \times cm^{-1}$)	λ_{em} (nm)	Φ[a]
Ethanol (51.9)	4.25×10^{-4}	465 333	1.43 1.08	none	none
Dichloromethane (41.1)	1×10^{-4}	452 326	15.2 11.2	513	very low [b]
Benzene (34.5)	1×10^{-4}	447 322	9.8 8.3	506	0.00100
Toluene (33.9)	1×10^{-4}	445 320	9.7 8.4	508	0.00112

[a]compared to quinine bisulfate (in 0.1N H_2SO_4, Φ=0.55); [b] 5.035×10^{-4} M

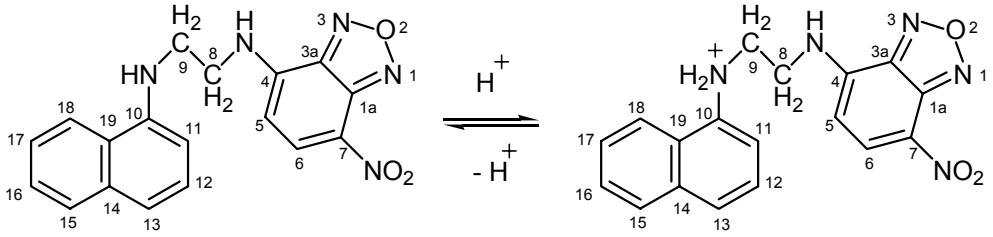
In compound **3c** there is an electron-acceptor NBD group (A) and a π-electron-donor moiety (D) represented by the α-naphthylamino group, linked together by a flexible ethylenediamino chain. An intramolecular D–A interaction will quench the fluorescence, but the protonation cancels the donor effect of the donor group.

By simulating the molecular geometry using the Hyperchem force field MM+,[48] it was possible to simulate the closed-sandwich geometry of **3c** as a consequence of the intramolecular D–A interaction. As seen in an earlier Section, NMR data (Table 2) confirm the structure of the salt **5** , and its geometry appears as an open structure without such an intramolecular D–A interaction (Scheme 3 and Fig. 4).

Table 9. The fluorescence of **3c** (1×10^{-4}M) in absolute ethanol with acids, λ_{ex} = 450 nm

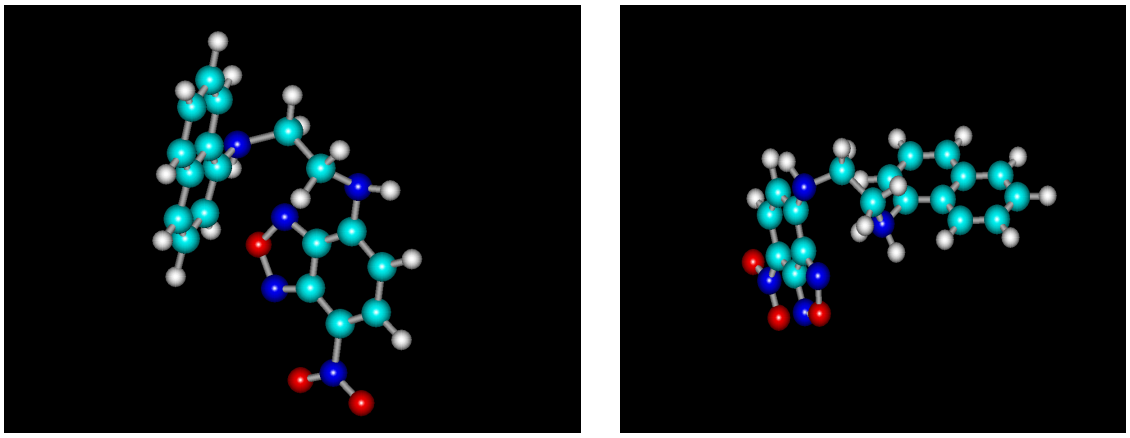
Acid	λ_{max} (nm)	E × 10^3 ($L\times mol^{-1}\times cm^{-1}$)	λ_{em} (nm)	Φ[e]
TFA[a] : EtOH[b] 1:1 v/v	449	10	521	0.00983
	318	4		
5×10^{-4} M pTSA[c] in ethanol	465	17.0	528	0.00222
	332	12.6		
CH_3COOH : EtOH[b] 1:1 v/v	465	20.2	534	0.00116
	330	15		
5×10^{-4} for N-Ph-Gly[d] in EtOH[b]	*465*	21.7	534	Very low[f]
	393 (sh)	25.3		

[a] TFA = trifluoroacetic acid; [b]Absolute EtOH; [c]pTSA = 4-toluenesulfonic acid, monohydrate; [d]N-Ph-Gly=N-phenylglycine; [e] compared to quinine bisulfate (in 0.1N H_2SO_4, Φ=0.55); [f] 6.51×10^{-4} M.



3c **5**

Scheme 3. The reversible protonation of **3c.**



3c **5**

Figure 4. Optimized geometries (with the MM+ program from Hyperchem) for the non-fluorescent compound **3c** and its conjugate acid **5** (Scheme 3).

Qualitative experiments with compound **3c** evidenced the fluorescence-enhancing effect of inorganic acids (e.g. HCl, H_2SO_4, H_3PO_4, HPO_3, $H_4[Si(W_3O_{10})_4]$) or organic acids (e.g. bile acids, nicotinic acid, sulfanilic acid, salicylic acid, tannic acid). Compound **3c** does not become fluorescent in the presence of benzoic, ascorbic, or caprilic acids, as well as α-amino acids (i.e. leucine, alanine, phenylalanine, glycine, thyrosine, glutamic acid, arginine, ornitine).

Fluorescence of compound 3f

It was shown earlier that compounds **3e, 3f,** and **3g** have the highest fluorescence in the series examined in this report. The hydrophobicity of these compounds decreases in the order **3e** > **3g** > **3f**. The last compound is actually amphiphilic due to the presence of the hydrophobic NBD moiety, and the hydrophilic tris(hydroxymethyl) group. We examined the behavior of the fluorescence of **3f** in aqueous ethanol as a function of the ethanol concentration. As shown in Fig. 5, the fluorescence intensity raises markedly with an increasingly higher ethanol content (about 20 times from 20% to 96% ethanol).

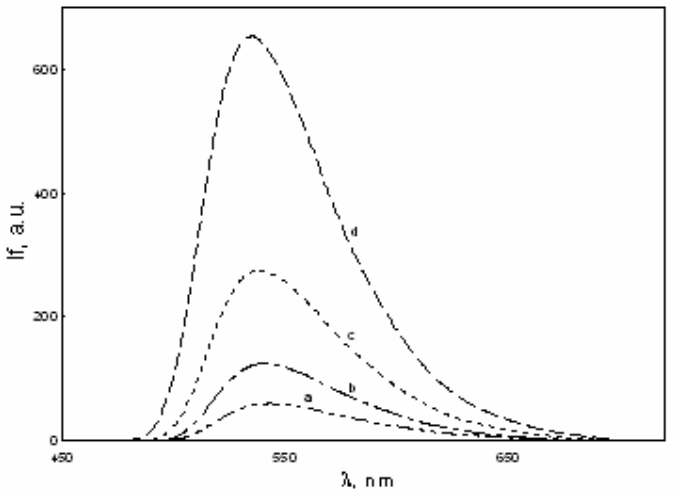


Figure 5. Change of the fluorescence intensity for compound **3f** (conc. = 1.4×10^{-3} M, λ_{ex} = 450 nm) in aqueous ethanol: a = 20% ethanol–water; b = 40% ethanol–water; c = 60% ethanol–water; d = 96% ethanol–water.

One can explain this behavior by the solvent polarity [49-53] and/or by assuming that **3f** may form molecular aggregates like „multivalent molecules".[54-58] Thus, compound **3f** may be useful as a *fluorescent probe* for exploring how the stronger non-covalent interactions (hydrogen bonds, hydrophobic interactions, donor-acceptor or charge transfer interactions) behave for biomolecules such as glycoproteins, glycolipids, lectins. More generally, all strongly fluorescent compounds **3e**, **3f**, **3g** may be useful as molecular fluorescent probes for antibody-antigen biochemical species that manifest affinity for 2,4-dinitrophenyl groups, which are similar to the NBD moiety.[41,59]

Conclusions

The present study was undertaken in order to obtain new 4-amino-7-nitro-NBD derivatives **3b**–**3g** by reacting NBD-Cl with corresponding amines. The known 4-anilino derivative **3a**, which is weakly fluorescent, was the reference compound. With a benzo-crown structure, **3b** has ionophoric character. The weakly fluorescent N-α-naphthyl-N'-NBD-ethylenediamino derivative **3c** becomes intensely fluorescent on treatment with strong acids, as the result of a change in geometry that cancels the intramolecular fluorescence quenching. The weakly fluorescent paramagnetic derivative **3d** with an amino-TEMPO nitroxide group becomes intensely fluorescent on reduction with ascorbic acid yielding the corresponding hydroxylamine derivative. Compounds **3e** with an α-picolyl group and **3g** with a hydroxylamino group are strongly fluorescent. Derivative **3f** with a tris(hydroxymethyl) group has an amphiphilic character and may be useful as a molecular probe for studying emulsions and micelles. Other derivatives (**3b**–**3d**) may be useful as biochemical fluorescent probes.

Experimental Section

General Procedures. Chemicals (amines **2a**–**2g**) and NBD-Cl (**1**) were Aldrich commercial products. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded with a Varian Gemini 300BB spectrometer at 300 MHz for protons and 75 MHz for ^{13}C. Electronic absorption spectra were recorded with a Perkin-Elmer Lambda UV-Vis spectrophotometer, and fluorescence with a Perkin-Elmer 204 spectrofluorimeter using an excitation lamp (Xe, 150 W) interfaced with the computer, allowing a pre-established data reading time of 0.5 s. EPR spectra were recorded using a Jeol JES FA100 spectrometer. IR spectra were recorded with a Bruker FTIR spectrophotometer Model Vertex 70, using ATR technique. Melting points have been recorded in open capillary with Electrothermal's IA 9000 Series of digital melting point instruments.

Synthesis of compounds 3a-g. General procedure

The 4-chloro-7-nitro-benzofurazan **1** was treated with amines **2a**–**2g** in the following molar ratio: 1:1 for **3b, 3d, 3e;** 2:1 for **3f, 3g** ; 1:2 for **3c** and a large excess (about 11:1) for **3a**. The reaction medium (about 10 mL/gram of **1**) was: acetonitrile for **3a**, methanol for **3b, 3c, 3e,** ethanol for **3d, 3f**, and methanol:water 1:1,v/v for **3g.** An excess (about 3 mol/1 mol of **1**) of sodium hydrogen carbonate was used for **3b-e** and **3g**. The mixture was stirred for one hour for **3c, 3g**, two hours for **3d, 3f**, and 24 hrs for **3a**, **3b**, and **3e** (at room temperature for **3a, 3b, 3e**, or at 50˚C for **3c, 3d, 3f, 3g**). The products **3a-g** were isolated from the reaction mixture as follows:

(i) For **3a** – **3d** and **3g** after filtration through a G3 glass filter, the solution was shaken with a tenfold volume of 1N hydrochloric acid and extracted with methylene chloride. The organic phase was dried over anhydrous sodium sulfate, and the solution was concentrated under reduced pressure. Compound **3a** was obtained in pure state (confirmed by TLC, silica gel Merck GF_{254},

CH_2Cl_2, once). Compounds **3b**, **3c**, **3d**, and **3g** were isolated from the concentrated solution similarly by repeated preparative TLC, and was purified TLC using silica gel Merck GF_{254} and the following elution solvents: for **3b**, CH_2Cl_2:MeOH 9:1 (v/v), once; for **3c** and **3g**, CH_2Cl_2: twice; for **3d**, CH_2Cl_2:MeOH 9.9:0.1 (v/v), once.

(ii) For **3e**, the precipitate retained after filtration through a G3 glass filter was purified by preparative TLC using silica gel Merck GF_{254} with CH_2Cl_2 (three times).

(iii) For **3f**, two consecutive extractions were performed: first with CH_2Cl_2 till the organic phase remained colorless, then with ethyl acetate till the organic phase was no longer fluorescent. The organic phase was dried over anhydrous sodium sulphate, the solution was concentrated under reduced pressure and the product was obtained in pure state by preparative TLC using silica gel Merck GF_{254} with CH_2Cl_2:MeOH 9:1 (v/v), three times.

4-Amino-7-nitro-*N*-phenyl-2,1,3-benzoxadiazole (3a). 95% yield, red solid, m.p. 151-152ºC (lit. m.p. 150ºC [1] and 152-153ºC [12]); Anal.: Calcd. for $C_{12}H_8N_4O_3$: C 56,26; H 3.15; N 21.87; found C 56.24; H 3.10; N 21.81; IR (ATR), cm^{-1}: 1554 (NO_2), 3289 (NH).

4-(4'-Aminobenzo-18-crown-6)-7-nitro-2,1,3-benzoxadiazole (3b). 72% yield, red solid, m.p. 147-148ºC; Anal.: Calcd. for $C_{22}H_{26}N_4O_9$: C 53,88; H 5.34; N 11.42; found C 53.85; H 5.33; N 11.38; IR (ATR), cm^{-1}: 1566 (NO_2), 2912 (CH_2), 3520 (NH).

***N*-1-Naphthyl-*N'*-(7-nitro-2,1,3-benzoxadiazole-4-yl)ethane-1,2-diamine (3c).** 68% yield, red-brown solid, m.p. 195-196ºC; Anal.: Calcd. for $C_{18}H_{15}N_5O_3$: C 61,88; H 4.32; N 20.04; found C 61.85; H 4.30; N 20.00; IR (ATR), cm^{-1}: 1574 (NO_2), 2924 (CH_2), 3327 (NH). On treatment with the acids mentioned in the text and Table 2, a strong fluorescence due to the salt **5** is observed.

4-(Amino-2',2',6',6'-tetramethylpiperidinyloxy)-7-nitro-2,1,3-benzoxadiazole (3d). 16% yield, red-brown solid, m.p. 235-236ºC; Anal.: Calcd. for $C_{15}H_{20}N_5O_4$: C 53,88; H 6.02; N 20.94; found C 53.85; H 6.00; N 20.88; IR (ATR), cm^{-1}: 1313 (N-O·), 1577 (NO_2), 2932, 2980 (CH_2, CH_3), 3215 (NH).

7-Nitro-*N*-(pyridine-2-yl-methyl)-2,1,3-benzoxadiazole (3e). 52% yield, yellow-reddish solid, m.p. 194-195ºC; Anal.: Calcd. for $C_{12}H_9N_5O_3$: C 53,14; H 3.34; N 25.82; found C 53.11; H 3.33; N 25.77; IR (ATR), cm^{-1}: 1581 (NO_2), 2920 (CH_2), 3293 (NH).

2-(Hydroxymethyl)-2-[(7-nitro-2,1,3-benzoxadiazole-4-yl)amino]propane-1,3-diol (3f). 22% yield, dark brown solid, m.p. 216-217ºC; Anal.: Calcd. for $C_{10}H_{12}N_4O_6$: C 42,26; H 4.25; N 19.71; found C 42.23; H 4.21; N 19.67; IR (ATR), cm^{-1}: 1576 (NO_2), 2923 (CH_2), 3277, 3354 (OH).

4-Amino-*N*-hydroxy-*N*-methyl-7-nitro-2,1,3-benzoxadiazole (3g). 17% yield, brown-reddish solid, m.p. 235-236ºC; Anal.: Calcd. for $C_7H_6N_4O_4$: C 40,00; H 2.87; N 26.66; found C 39.96; H 2.84; N 26.60; IR (ATR), cm^{-1}: 1579 (NO_2), 2920 (CH_3), 3292 (OH).

Reduction of compound 3d to 4 (Scheme 2)

A six-fold molar excess of ascorbic acid was added to the solution of **3d** in absolute ethanol under stirring at room temperature till TLC shows the disappearance of **3d** and the complete

formation of **4** (R_f **3d** = 0.907, R_f **4** =0.372, silica gel, CH_2Cl_2:MeOH 9.5:0.5 (v/v), detection by UV at 254 nm and 360 nm, Figure 3.

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