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Dedicated to Professor Mihaela Hillebrand on the occasion of her 85th anniversary

SYNTHESIS AND STRUCTURAL ANALYSIS OF THE NBD-DERIVATIVE OF THE AMINO-HEXANOIC ETHYL ESTER

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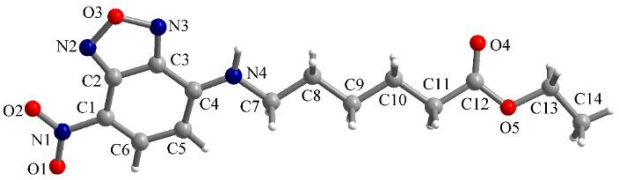
Starting from 6-aminohexanoic acid and nitrobenzozadiazole-chloride, the corresponding derivative was obtained, which was further reacted with ethanol either by DCC/DMAP coupling reaction or by activation with thionyl chloride, thus yielding the ester, namely ethyl 6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)hexanoate. The characterization of this compound was performed by UV-Vis and fluorescence, NMR, IR, MS, and X-ray diffraction on single crystal.



INTRODUCTION

NBD-chloride (4-chloro-7-nitrobenzo-2-oxa-1,3-diazole) is a well-known non-fluorescent highly reactive reagent towards amines that convert them into luminescent derivatives. This process is commonly used from decades for labelling amino-acids, peptides and proteins, and was also extended to lipids.[1–5] A survey of the recent application of NBD derivatives showed that fluorescence microscopy benefits from the use of novel NBD derivatives.[6]

Usually, NBD-derivatives have shown remarkable increase in fluorescence intensity, making them valuable in various applications such as sensing and bioimaging. Due to the unique π-electron heteroatom structure and nitro electron-withdrawing group, NBD is electrophilic, easily reacting with amines or thiols functional groups by aromatic substitution reaction.[7]

For example, in order to address specific aspects regarding the molecular size and dynamics of NBD-cholesterol derivatives, the lateral diffusion characteristics using FRAP were studied.[8] Moreover, synthesis of molecular probes based on conjugation of NBD moiety with different substrates affords pH sensitive probes.[9]

Based on these literature data, our aim was to extend the derivatives of NBD that possess such important and interesting behaviour, like intense

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colour and fluorescence. As a result, a novel compound was synthesized and characterized by different means, as it is shown further.

RESULTS AND DISCUSSION

Synthesis

Some NBD-derivatives of the 6-aminohexanoic acid esters are known, like the *t*-butyl or methyl ones;[10,11] however, the ethyl ester was not found in the literature, and therefore this compound was next synthesized and structurally described. NBD-derivatives are important in biological applications.[12,13]

As starting material in the synthesis was used the NBD derivative of the 6-aminohexanoic acid, which can be converted into the ethyl ester following two variants (Fig. 1). Thus, in the first one, the acid group is activated by the coupling reagent dicyclohexylcarbodiimide (DCC), while dimethylaminopyridine (DMAP) acts as a catalyst; consequently, in the presence of ethanol, the ester is obtained in moderate yields (40%). In the second variant, the acid is converted first to the corresponding acyl chloride by reaction with thionyl chloride, followed by the reaction with ethanol, yielding in very good yields the ester (90%). In both cases, a further purification by column chromatography is advised, leading to the analytical samples that were studied by different spectroscopic means. Slow evaporation of the solution also afforded crystals that were suitable for X-ray analysis. Although the DCC/DMAP synthesis has lower yields, the crude reaction mixture contained less by-products (as TLC showed), which can be beneficial in term of separation of the desired compound.

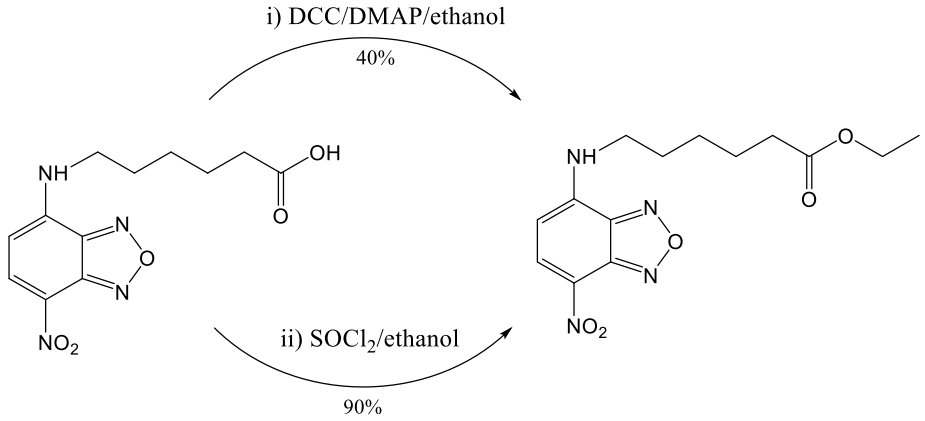


Fig. 1 – Synthesis of the ethyl ester following two variants.

Structural analysis

The solid-state structure of the title compound was determined by X-ray diffraction on single crystal. This compound crystallizes in the orthorhombic *Pba2* space group with one molecule in the asymmetric unit (Fig. 2). Within the 4-amino-7-nitrobenzofurazan fragment, the nitro group is practically coplanar with benzofurazan fragment. The dihedral angle between the mean planes benzofurazan and nitro groups is 1.2º. In the nitro-benzofurazan fragment the N-O bond lengths are: N1–O1 = 1.222(3), N1–O2 = 1.222(3), N2–O3 = 1.393(3) and N3–O3 = 1.371(3) Å. The C–N bond lengths are: C1–N1 = 1.435(3), C2–N2 = 1.321(3), C3–N3 = 1.311(3), C4–N4 = 1.337(3) and C7–N4 = 1.456(3) Å.

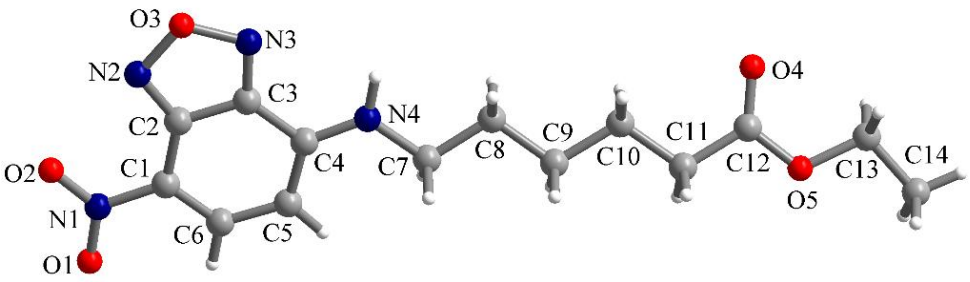


Fig. 2 – View of the molecular structure of the compound along with the atoms labelling scheme.

The secondary amino group of each molecule is involved in hydrogen interactions with the ester group belonging to a neighbouring molecule generating supramolecular double piles running along the crystallographic *c* axis (Fig. 3). The H1N atom interacts with the O4' atom of an ester group (symmetry code:' = 2–x, –y, –1+z). The distances for the hydrogen interactions are: (N4)H1N··· O4' = 2.154 Å and the corresponding angles are: N4–H1N··· O4' = 169.1 °. The interactions within a pile are reinforced by p–p contacts established by the nitro-benzofurazan fragments (3.44–3.51 Å). The overlapping mode is presented in the inset of Fig. 3.

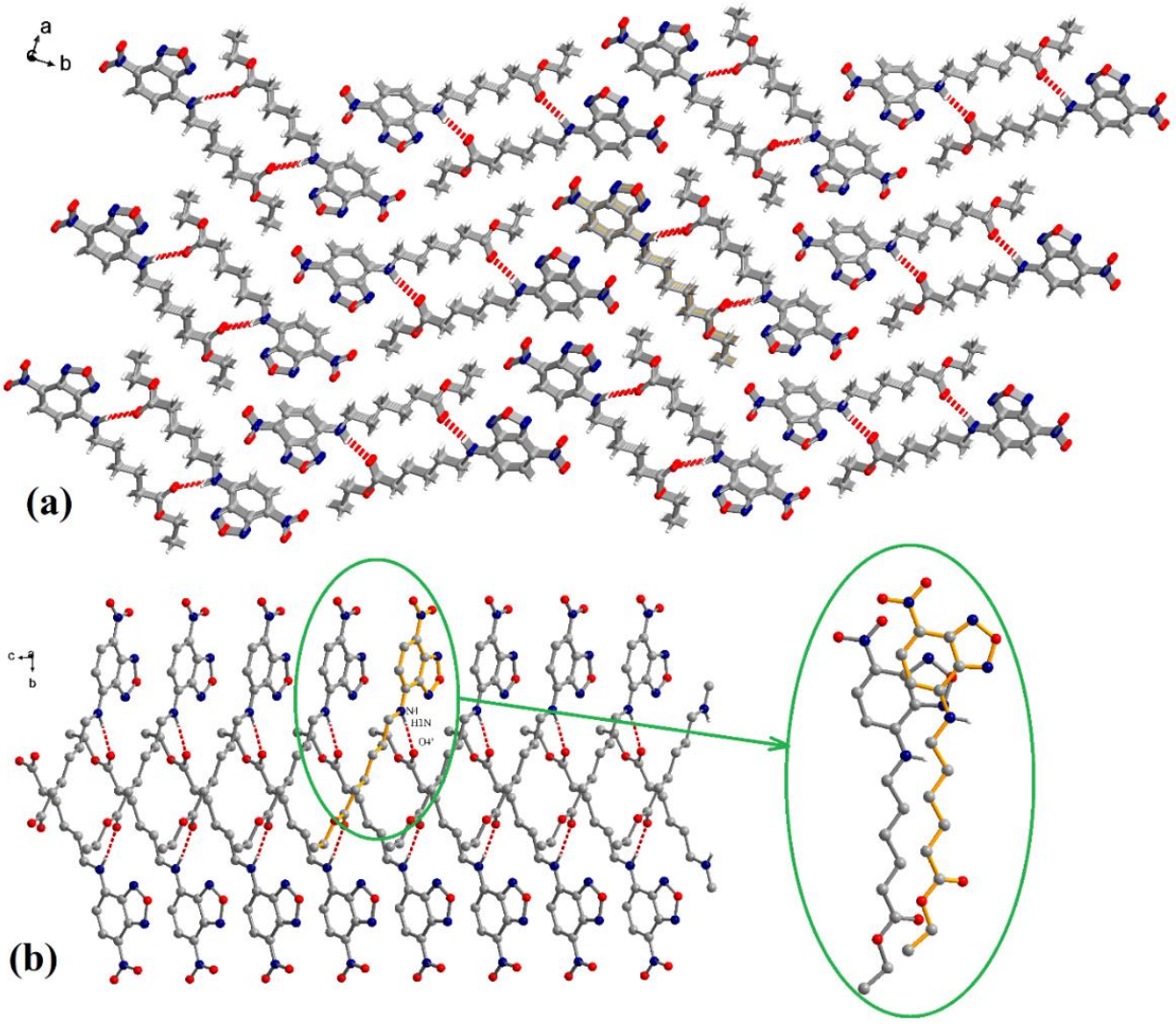
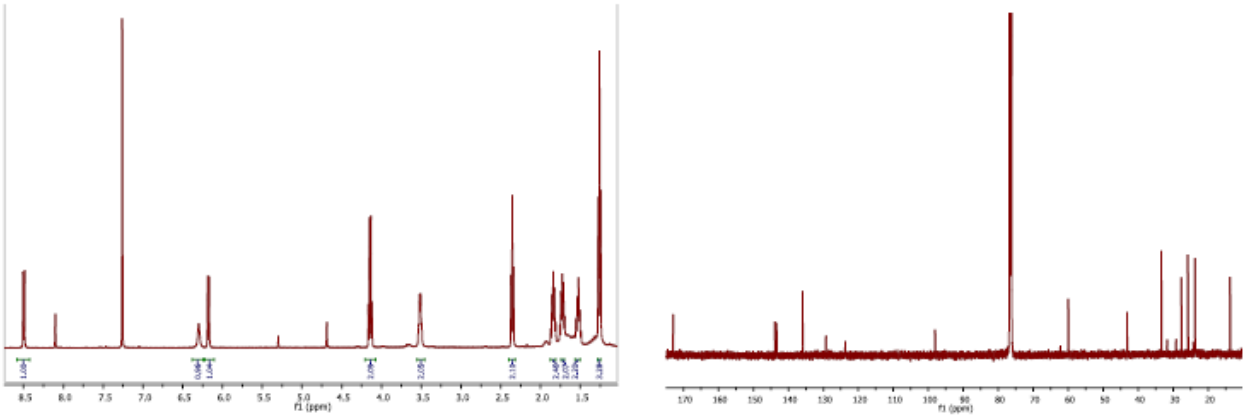


Fig. 3 – Views of the packing diagrams along the crystallographic *c* (a) and *a* axes (b).

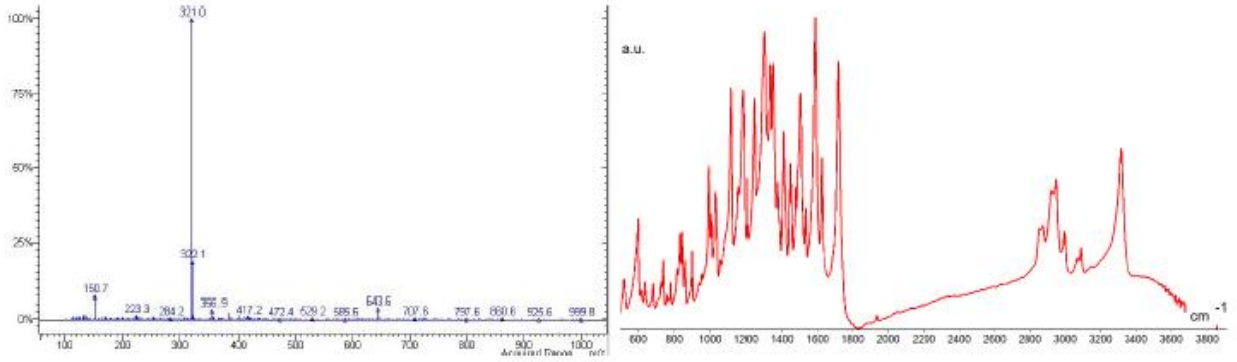
The ^{1}H-NMR spectrum (Fig. 4a) shows all relevant peaks with the corresponding integrals and splitting. Thus, *H*-nuclei from the NBD-moiety appears as doublets at 8.50 and 6.18 ppm, –O–C*H*$_2$– as quartet at 4.14 ppm, while –C*H*$_3$ group as triplet at 1.33 ppm. In the ^{13}C-NMR spectrum (Fig. 4b), different types of signals are present, according to the molecular structure, the signal at 172.90 ppm being relevant for the carbonyl group.

The ESI-MS spectrum recorded in the negative mode showed the corresponding molecular peak $[M-H]^-$ at 321 m/z (base peak, 100% intensity, Fig. 5a), confirming the structure of the compound. Supplementary information can be found in the IR spectrum (Fig. 5b). Thus, the N–H stretching vibration appears at 3313 cm^{-1}, aromatic and aliphatic C–H around 3100 cm^{-1} and 2900 cm^{-1}, respectively. The most intense peak is recorded for the carbonyl group at 1718 cm^{-1}, while nitro group are recorded about 1550 and 1350 cm^{-1}.



(a) (b)

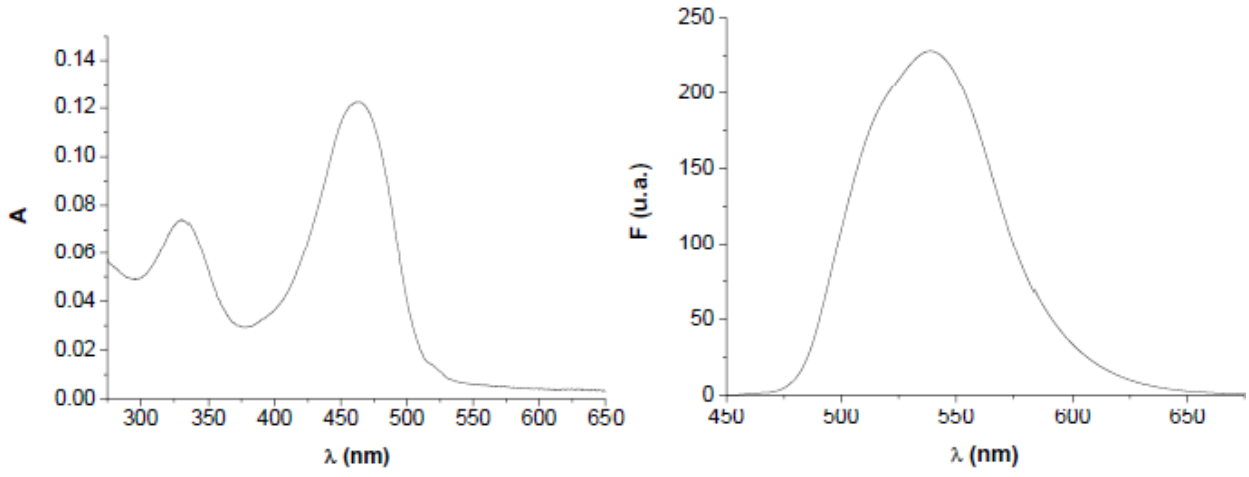
Fig. 4 – a) ^{1}H–NMR and b) ^{13}C–NMR spectra recorded in $CDCl_3$.



(a) (b)

Fig. 5 – (–) ESI-MS (a) and IR (b) spectrum of the title compound.

As mentioned before, NBD-derivatives of aliphatic amines have yellow-red intense colour and have shown bright strong fluorescence. UV-Vis and fluorescence spectra recorded in ethanol for our compound are shown in Fig. 6. Thus, the newly synthesized derivative showed the most intense absorption band at 463 nm (a smaller one being present around 330 nm), while excitation at the same wavelength led to an emission band at 539 nm (with a shoulder at 515 nm).



(a) (b)

Fig. 6 – UV-Vis (a) and fluorescence spectra (b) recorded in ethanol.

EXPERIMENTAL

Materials and methods

All chemicals, solvents, and materials were purchased from Merck or Chimopar and used as received. IR spectra (ATR) were recorded on a Jasco FTIR 4700 spectrophotometer. NMR spectra were obtained using a Bruker Fourier 500 MHz instrument, employing $CHCl_3$-d1 as solvent. UV-Vis spectra were recorded in ethanol as solvent using a Jasco V−630 (10 mm quartz cell). The MS spectra were recorded on a Varian 310 MS LC/MS/MS triple quadrupole mass spectrometer fitted with an electrospray ionization interface (ESI). The synthesis of the starting material, 6-((7-nitrobenzo[c][1,2,5]oxadiazol-4 yl)amino)hexanoic acid, was achieved as literature data showed.[14,15]

Synthesis of ethyl 6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)hexanoate.

Variant I. 298 mg of the NBD derivative of 6-amionohexanoic acid (1 mmol) was dissolved in 75 mL DCM to which were added 310 mg DCC (1.5 mmol), few mg of DMAP and 0.5 ml ethanol, and the mixture left at room temperature for 3 days. The reaction mixture was then washed with a diluted aqueous solution of sodium hydrogen carbonate, followed by a solution of diluted hydrochloric acid, and the organic phase separated, dried over anhydrous sodium sulphate, and the solvent removed under vacuum. The residue was chromatographed on a silica gel column using DCM as eluent. Yields around 130 mg (40%).

Variant II. 298 mg of the NBD derivative of 6-amionohexanoic acid (1 mmol) was suspended in 25 mL DCE to which were added two drops of DMF and 0.5 mL thionyl chloride, and the mixture refluxed for one hour, followed by addition of 10 mL of ethanol. Next day the work up was done as in variant I. Yields 290 mg (90%).

$C_{14}H_{18}N_4O_5$ MW 322. R_f 0.18 (DCM/silica gel). M. p. 134–136 ºC. 1H–NMR (500 MHz, $CHCl_3$-d1, δ ppm, J Hz): 8.50 (d, 1H, NBD, 9); 6.30 (s, 1H, NH); 6.18 (d, 1H, NBD, 9); 4.14 (q, 2H, OCH_2, 7); 3.53–3.49 (m, 2H, N–CH_2); 2.36 (t, 2H, $COCH_2$, 7.5); 1.87–1.81 (m, 2H, CH_2); 1.76–1.69 (m, 2H, CH_2); 1.55–1.49 (m, 2H, CH_2); 1.26 (t, 3H, CH_3, 7.5). ^{13}C–NMR (125 MHz, $CHCl_3$-d1, δ ppm): 172.9; 143.8; 143.4; 143.2; 135.9; 129.2; 1213.7; 60.0; 43.1; 33.4; 27.7; 25.8; 23.7; 13.7. IR (KBr, cm^{-1}): 3313; 3088; 3058; 2995; 2947; 2921; 2872; 1718; 1584; 1500; 1445; 1347; 1298; 1242; 1189; 1121; 992; 842; 595; 516.

X-ray crystallographic analysis

X-ray diffraction measurements for the title compound were performed on a Rigaku XtaLAB Synergy-S diffractometer operating with Mo-Kα (λ = 0.71073 Å) micro-focus sealed X-ray tube. The structure was solved by direct methods and refined by full-matrix least squares techniques based on F^2. The non-H atoms were refined with anisotropic displacement parameters. Calculations were performed using SHELX-2018 crystallographic software package. A summary of the crystallographic data and the structure refinement for the new compound are given in Table 1. CCDC reference number: 2545056.

Table 1

Crystallographic data, details of data collection and structure refinement parameters

Compound	
Chemical formula	$C_{14}H_{18}N_4O_5$
M (g mol^{-1})	322.32
Temperature, (K)	293(2)
Wavelength, (Å)	0.71073
Crystal system	*Orthorhombic*
Space group	*Pba2*
a (Å)	11.1445(8)
b (Å)	31.545(2)
c (Å)	4.3294(3)
α (º)	90
β (º)	90
γ (º)	90
V ($Å^3$)	1522.00(18)
Z	4
D_c (g cm^{-3})	1.407
μ (mm^{-1})	0.109
F(000)	680
Goodness-of-fit on F^2	0.988
Final R_1, wR_2 [I>2σ(I)]	0.0332, 0.0803
R_1, wR_2 (all data)	0.0440, 0.0850

CONCLUSIONS

Using two synthetic pathways, the new compound ethyl 6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino) hexanoate was obtained and characterized by NMR, MS, IR, UV-Vis, and fluorescence. The structural analysis was completed by X-ray diffraction on single crystal. The new ester is a bright yellow coloured compound that also possess a strong fluorescence, making it suitable for implication in the study of different chemical or biological systems.

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