Russian Journal of Bioorganic Chemistry, Vol. 31, No. 3, 2005, pp. 297–299. Translated from Bioorganicheskaya Khimiya, Vol. 31, No. 3, 2005, pp. 331–334.

LETTERS TO THE EDITOR

A Synthesis of New Rigid Fluorescent Bichromophoric Probes for Studying Mechanisms of Donor–Donor Energy Migration

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Received December 29, 2004; in final form, January 13, 2005

Abstract—Three new fluorescent probes were synthesized for improving the method of studying donor–donor energy migration (DDEM). Each probe has two identical fluorescent 7-diethylaminocoumarin-3-carbonyl groups attached to a rigid bisteroid dodecacyclic spacer through additional inserts. In two probes, the inserts are β-Ala and *L*-Ser residues, which provide for a different nearest environment of the fluorophores. The third probe has identical β-Ala inserts.

Key words: DDEM, fluorescent bichromophoric probes, fluorescence spectra, synthesis

We had previously reported [1, 2] the synthesis of a series of fluorescent bichromophoric probes intended for studying the migration of excitation energy between fluorophores in model systems (for a review, see [3]).[2]

The development of the DDEM method required a consideration of conditions at which energy migrates between the fluorophores placed in different environment. It is these situations that take place in the majority of biological and model systems. The energy migration is partially reversible in such asymmetric (from the photophysical point of view) systems. The PDDEM model has been suggested for its description [4]. The use of this model and the probes synthesized [1, 2] helped measure some membrane parameters, in particular, the thickness of liposomal bilayer in which the same (rhodaminyl or fluoresceinyl) fluorophores were at its different sides [5, 6].

Sophisticated bifluorophoric probes, in which the same fluorophore is primarily present at two different surroundings, i.e., in the neighborhood of grouping with different polarity, are necessary for a further improvement of the PDDEM method. The study of PDDEM in such probes embedded in various media permit the establishment of regularities of the phenomenon under study.

We describe here the synthesis of such probes (see the scheme). Each of compounds (**VII**) and (**VIII**) has two equal fluorophores, DEAC residues, attached to amino groups of different spacers: β-Ala residues (in both probes), and a *O*-acetylserine [in probe (**VII**)] or a Ser residue [in probe (**VIII**)]; in turn, they are situated at two opposite sides of the rigid decacyclic system (the so-called bisteroid [7]). Therefore, equal fluorophores have the different nearest surroundings represented by an additional acetoxy (**VII**) or a hydroxyl group (**VIII**). In addition, probe (**V**) was obtained as a comparison standard; the two fluorophores attached to β-Ala spacers are there in equal environment. Strictly speaking, some differences also are here, since the bisteroid molecule is asymmetric in its central part; however, these differences are so insignificant that show no influence on the photophysical properties of fluorophores [1].

The choice of DEAC residue as a fluorophore was dictated by the fact that DEAC easily acylates amino groups and the resulting derivatives retain good quantum yields [8]. The fluorescence parameters of DEAC group are very sensitive to the polarity of environment, as in the case of the majority of other polar fluorophores. Moreover, the position of the excitation maximum of DEAC derivatives (420–425 nm) allows the use of DEAC group for the acception of excitation energy of protein fluorophores, which broadens the potentialities of DEAC probes in studying lipid–protein interactions. A number of laboratories are studying the modification of DEAC with the purpose of design of new fluorophores and probes on their basis (see [9] and references therein).

The distance between fluorophores directly bound to the bisteroid is approximately 20 Å [7]. Additional spacers, the β-Ala and Ser residues, increases this distance to ~30 Å (in the extended conformation of probe). This value is close to an average thickness of membrane bilayer. Therefore, both fluorophores of the probe would be located in the region of polar head groups on the opposite sides of membrane, when bifluorophoric probes (**V**), (**VII**), and (**VIII**) were normally oriented to

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[2] Abbreviations: BOP, (benzotriazol-1-yloxy)-tris(dimethylamine)phosphonium; and DDEM, donor–donor energy migration; DEAC, 7-diethylaminocoumarin-3-carboxylic acid; PDDEM, partial donor–donor energy migration.

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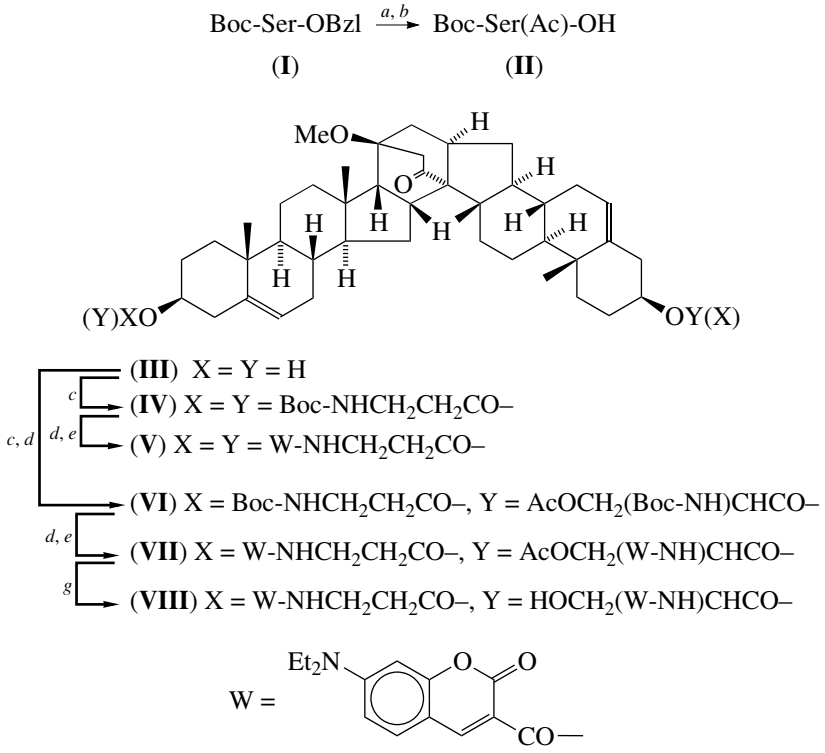


a, Ac_2O/Et_3N; *b*, H_2/Pd; *c*, Boc-$HN(CH_2)_2COOH$/DCC/4-pyrrolidinopyridine; *d*, TFA; *e*, DEAC/BOP/$EtPr^i_2N$; *f*, (**II**)/DCC/4-pyrrolidinopyridine; *g*, K_2CO_3/MeOH.

Scheme 1.

the bilayer. We have previously shown that a bichromophoric probe constructed from a nonpolar spacer and polar fluorophores is disposed in membrane exactly in such a way [5].

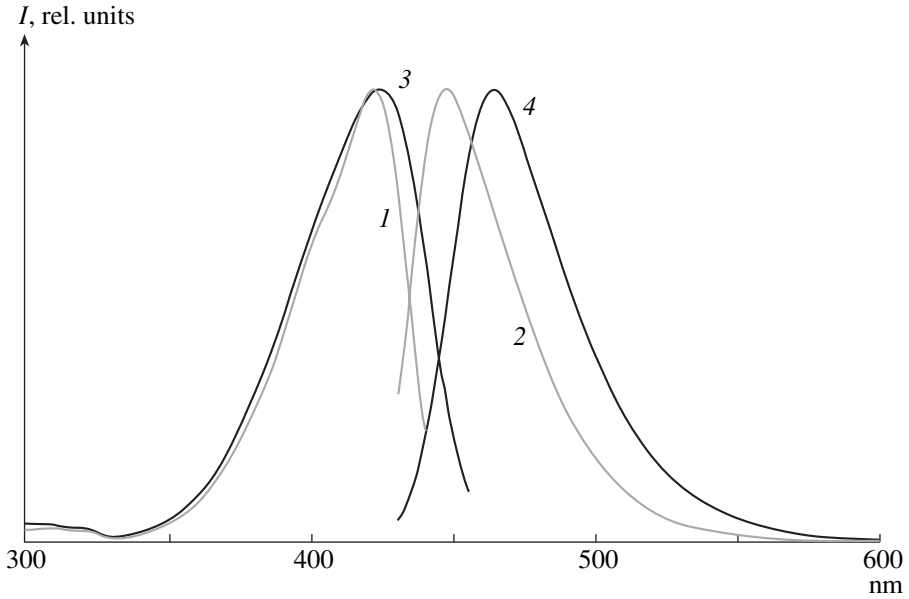
The DEAC probes were synthesized by classical methods. The first probe (**V**) was obtained by acylation of bisteroid (**III**) with *N*-Boc-alanine using the carbodiimide method. The resulting diester was treated with TFA to remove Boc protection, and the produced diamine was acylated with DEAC in the presence of BOP (Fluka) as a condensing reagent by the method, which was successfully used for the synthesis of other fluorescent probes [2] (Scheme 1).

Other probes (**VII**) and (**VIII**) were prepared as follows. *N*-Boc-*L*-serine benzyl ester (**I**) (NovaBiochem) was converted into *N*-Boc-*O*-Ac-*L*-serine (**II**) by acetylation (Ac_2O/Et_3N) and subsequent hydrogenolysis over palladium catalyst in dioxane. Bisteroid (**III**) was first acylated with *N*-Boc-β-alanine (1.3 equiv) as described above. Monoester of *N*-Boc-β-alanine and bisteroid was isolated from a mixture of the reaction products and, then, acylated with (**II**). The resulting diester (**VI**) was converted into probe (**VII**) by the successive treatment with TFA and DEAC (Molecular Probes) + BOP. The nearest surrounding of one of the fluorophores in this probe always differs from that of the other fluorophore due to the immediate closeness of acetoxy group.

Probe (**VIII**) was obtained from (**VII**) by a basic methanolysis of acetoxy group. Hydrogenolysis, which is successfully used for deacetylation in carbohydrate chemistry, was not suitable in this case because of the presence of keto group in the spacer. A use of methanol and potassium carbonate for the alcoholysis was insufficiently selective in respect of acetoxy group: it resulted in a marked cleavage of ester bonds between the β-Ala and Ser residues with bisteroid. The yield of (**VIII**) was about 50% at this stage.

Note that the isolation of DEAC-labeled compounds (**V**), (**VII**), and (**VIII**) is a problem, since the chromatography of these compounds belonging to bis-tertiary aromatic amines requires acidic eluents; however, such eluents (containing, e.g., TFA) can induce alcoholysis of ester bonds. For this reason, we did not use the reverse phase chromatography for the isolation of probes (**V**), (**VII**), and (**VIII**). Instead, we carried out the normal phase chromatography using the chloroform–ethyl acetate–CH_3COOH mixtures as eluents.

Probe (**VII**) was isolated in the form of two compounds with a close mobility on normal phase (silica gel) and the same mobility on the RP18 reversed phase. Both compounds had almost identical spectral charac-



(*1*, *3*) Excitation spectra at λ_{em} 465 nm and (*2*, *4*) emission spectra at λ_{ex} 415 nm of probe (**VIII**) in (*1*, *2*) chloroform and (*3*, *4*) ethanol at ~1 μM concentration and temperature 20°C.

teristics (see below). These compounds are presumably stereomers arisen from the introduction of a chiral *L*-Ser residue into the molecule of probe. Strictly speaking, there should be four stereomers, since the bisteroid spacer is also chiral. Therefore, the issue of the structure of isolated isomers of probe (**VII**) and the degree of their homogeneity still remains open. Probe (**VIII**) was obtained from one of the isolated isomers of (**VII**).

Structures of the compounds were confirmed by mass spectrometry with fast atom bombardment (FAB) and chemical ionization (CI) and by ^{1}H NMR spectroscopy. The most important diagnostic parameter of the NMR spectra was a ratio between the intensities of singlet signals of angular methyl groups of bisteroid at δ 0.67 and 0.84 ppm, as well as its *O*-methyl group (δ ~ 3.30 ppm) and the intensities of separate signals of aromatic protons of DEAC group in the region of δ 6.4–8.6 ppm (in $CDCl_3/CD_3OD$).

The UV spectra of probes (**V**), (**VII**), and (**VIII**) in ethanol are similar to the spectrum of DEAC, except for their shifts to the long-wave region. All probes had λ_{max} 420 nm, whereas the acid DEAC-OH exhibits λ_{max} 392 nm (in ethanol). The excitation spectra of (**V**), (**VII**), and (**VIII**) have a single maximum at 422–425 nm both in apolar (chloroform) and polar (ethanol) solvent [spectra *1* and *3* in the figure; the spectra of probe (**VIII**) are given]. This maximum corresponds to that of the absorption spectrum. A position of the maximum of emission spectrum depends on the polarity of environment: ~450 nm in chloroform and ~465 nm in ethanol (spectra *2* and *4*, respectively). These parameters of DEAC probes and the acceptable values of their quantum yields (0.2–0.4, preliminary data) allows us to think that probes (**V**), (**VII**), and (**VIII**) would find a proper application in the experiments aimed to a development of DDEM model.

ACKNOWLEDGMENTS

This work was supported by Russian Foundation for Basic Research, project nos. 00-04-48416 and 03-04-48420.

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