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ScienceDirect
Dyes and Pigments 74 (2007) 404–409
DYES and PIGMENTS
www.elsevier.com/locate/dyepig

Synthesis and characterisation of new phosphonate labelled cyanines

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Received 12 February 2005; accepted 1 March 2006
Available online 3 May 2006

Abstract

We present the synthesis of new *N*-1,5 phosphosubstituted pentamethine cyanine dyes in high yields. The compounds are obtained from original *N*-phosphonates quaternary heterocyclic ammonium salts, with an activated methyl group in the 2-position. Moreover, the aqueous solubility of these new dyes is emphasized. The spectroscopic parameters of the new compounds are described together with a preliminary study of divalent cations complexation in fluorescence.

Keywords: Cyanine dyes; Phosphorus ligands; Spectroscopic characterisation; Fluorescence; Complexation

1. Introduction

The synthesis [1,2] and the reactivity [3,4] of cyanine dyes were widely studied since years. They present numerous applications in large areas (chemistry, physics, materials …). For our part, we are particularly interested in their biological applications. They are often used as fluorescent probes in the investigations of biological materials, to study the structure of DNA and its detection in cells [5]. They have also many biomedical applications like disease diagnosis and prevention or treatment [6].

The synthesis of phosphorus podands labelled by a cyanine probe and the fluorescence enhancement with calcium or magnesium complexation were described in a previous paper [7]. This initial work showed that cyanines are interesting fluorescent transmitters to give a macroscopic quantified response to the presence of biological cations. Our actual goal is to develop new phosphorus functionalized cyanine dyes in this series which can be used as biological probes. The searched enhancements concern mainly the introduction of new phosphorus moieties, synthetic improvement, aqueous solubility and selectivity of the complexation.

Methods for the synthesis of new 1,5-substituted pentamethine cyanine dyes have been developed [8]. In the literature, there are many cyanines with *N*-alkyl substituent [9] but to the best of our knowledge, there is only one example with phosphonate moiety [10].

We present here the synthesis of new functionalized ammonium salt precursors and the derived cyanines dyes together with their preliminary photophysical results.

2. Results and discussion

We describe first the synthesis of the *N*-phosphonate quaternary ammonium salt precursors of new cyanines dyes. The ammonium salts **1a**, **2a** and **3a** bearing a phosphonate moiety as *N*-substituent were obtained by a classical nucleophilic substitution reaction. Following the procedure adapted from Almeida and others [11], by heating under reflux a solution of the corresponding 2-methylheterocyclic base **1**, **2** and **3** with an excess of diethyl 3-bromopropyl phosphonate **a** in acetonitrile (Scheme 1), **1a**, **2a** and **3a** are obtained in high yield.

In the course of this work, we also studied the reactivity of diethyl 2-bromoethylphosphonate **b** in acetonitrile. With the

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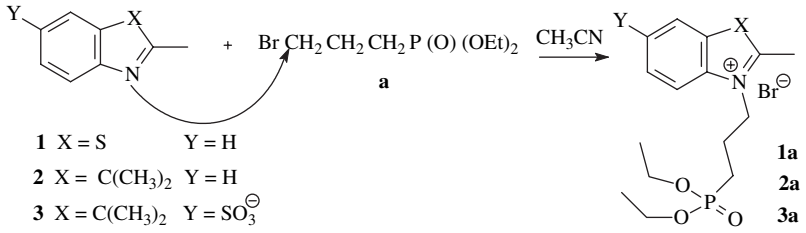
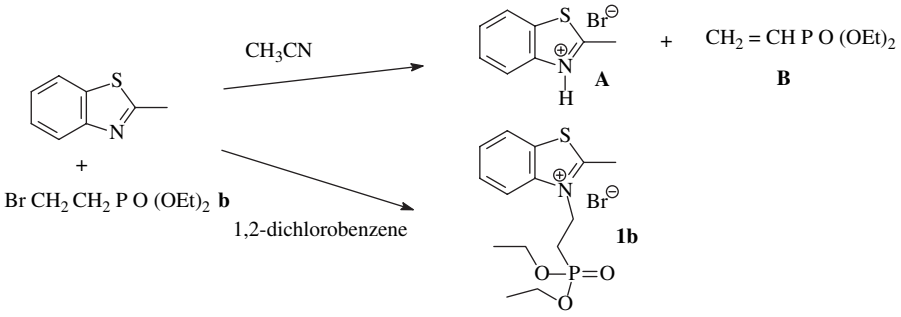
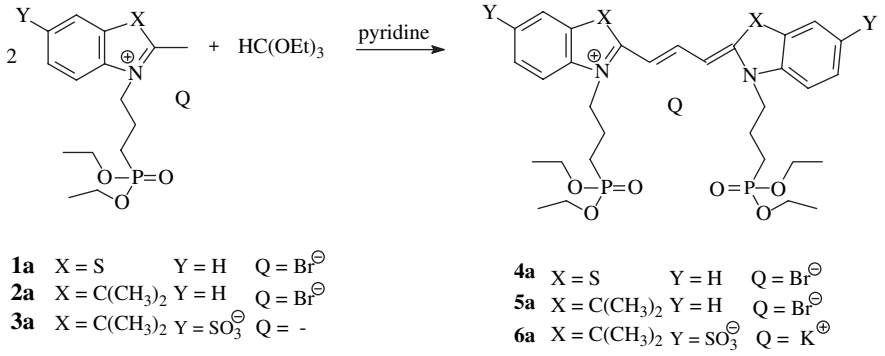
doi:10.1016/j.dyepig.2006.03.003



Scheme 1. Synthesis of ammonium phosphonate salts.



Scheme 2. Reactions with bromoethylphosphonate. Alternative pathways.



Scheme 3. Synthesis of cyanines **4**, **5** and **6**.

same operation conditions, the reaction proceeds differently following an elimination way of HBr and the formation of ammonium **A** and phosphonate **B**, compounds identified by NMR and mass spectra (Scheme 2, see Section 4). This is certainly due to a slight difference between acidity of the hydrogen atoms of the α methylene of the phosphonates **a** and **b**. With phosphonate **b**, these H atoms are quite more acidic due to the electro attractive effects of both the Br atom and phosphonate group, thus the benzothiazole acts as a base to abstract an α hydrogen with an elimination reaction of the bromine (probably E_2). With phosphonate, **a** there is an attenuation of the electro attractive effect of the Br atom due to the supplementary methylene group. In this case, the nitrogen atom of the ring attacks the terminal carbon of the diethyl 3-bromophosphonate (Scheme 1).

To favour the substitution mechanism with phosphonate **b**, we tried the reaction in dichlorobenzene at 80 °C during 24 h and these conditions were convenient for the obtention of ammonium **1b** (Scheme 2, see Section 4), this showed that the polarity of the solvent greatly influences the reactivity of **b**.

The electro attractive effect of phosphonate group is also evidenced when we compare the reactivity of diethyl 2-bromoethylphosphonate **b** with 3-bromopropionic acid one: the reaction of **1** with 3-bromopropionic acid leads to the expected *N*-quaternary ammonium salt, without formation of acrylic acid [11].

2.1. Cyanine dyes synthesis

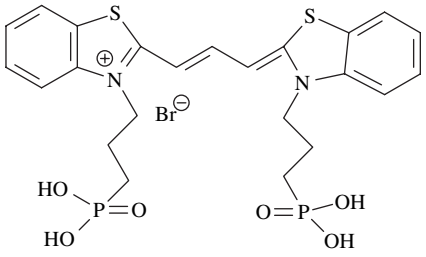
The process involves two steps:

(i) Synthesis of phosphorylated quaternary ammonium salt.
(ii) The condensation of two equivalents of the ammonium salt with ethylorthoformate $CH(OEt)_3$ in dry pyridine

(Scheme 3). The resulting salts obtained with good yields present a cyanine structure established by NMR, UV spectroscopy and mass spectra (see Section 4).

For a better solubility of the dyes in aqueous biological conditions and to enhance the complexing ability of the phosphorus moieties following the pH variations, we transformed the phosphonate moiety into phosphonic compound. The ammonium salt **1a** was dissolved in a mixture of acetic and hydrochloric acid (1/3) [12] and heated under reflux (48 h) until the complete transformation to phosphonic compound **1a′** (Scheme 4), characterized by NMR spectroscopy and mass spectra.

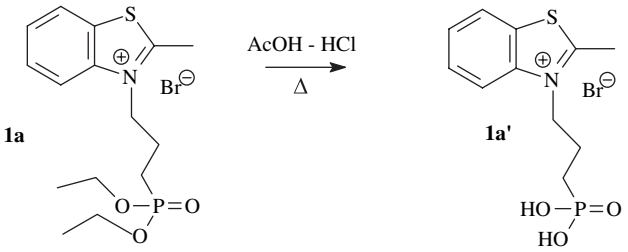
According to the previously described process, the corresponding cyanine **4a′** is obtained from ammonium **1a′** (see Section 4).



4a'

2.2. Calcium complexation

We present hereafter some preliminary results concerning the complexation of calcium by cyanine **4a**. According to the graph (Fig. 1), the addition of calcium perchlorate (concentration: 10^{-5} M) to a solution of 4.25×10^{-6} M of ligand **4a** in acetonitrile ($\lambda_{exc} = 520$ nm) leads to a reproducible increase in the fluorescence intensity as compared to the initial intensity of the cyanine alone.



Scheme 4. Conversion of phosphonate to phosphonic acid.

New experiments in different solvents and with other biological cations are currently in progress.

3. Conclusion

Some new phosphonate labelled cyanines were obtained with improved synthetic yields. They behave like potent fluorescent probes towards the complexation of biological cations. In their deprotonated form, they will represent a pH sensitive calcium probe and/or carrier with enhanced water solubility.

4. Experimental

4.1. General

All the reactants and chemicals were obtained from Sigma–Aldrich or prepared according to literature procedures. Solvents were of analytical grade or distilled over P_2O_5 in the case of acetonitrile. ^{1}H NMR spectra were recorded in CD_3CN or D_2O solutions on a Brüker ACP 250 (250.13 MHz) and ^{13}C NMR spectra on a Brüker ACP 300 (75.47 MHz). Chemical shifts are reported as δ values in ppm using TMS as an internal standard. Coupling constants (J) are given in Hz. Mass spectra (ESI positive and negative modes) were recorded in a Perkin–Elmer Sciex (API 365) or an Applied Biosystems (Q TRAP). UV–vis spectra are recorded on a Perkin–Elmer Lambda 9 Spectrophotometer at

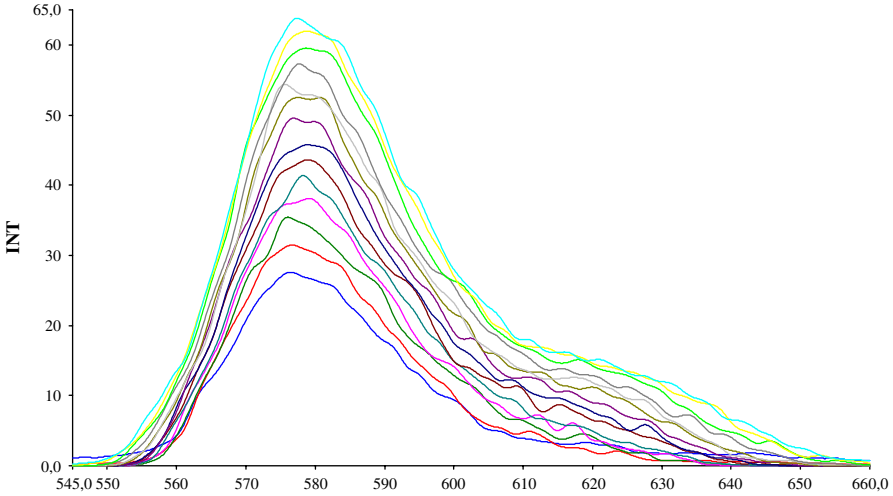


Fig. 1. Fluorescence intensity enhancement with successive addition of $Ca(ClO_4)_2$ in CH_3CN.

25 °C and fluorescence spectra on a Perkin–Elmer Luminescence Spectrometer LS 50 B at ambient temperature.

4.2. Synthesis of quaternary ammonium salts: **1a**, **2a**, **3a**

A solution of the heterocyclic compound [2-methylbenzothiazole (**1**), 2,3,3-trimethylindolenine (**2**) or 2,3,3-trimethylindoleninium-5-sulfonate (**3**)], synthesized by conventional Fisher indole synthesis [13] (1.0 mmol) and the bromopropionic phosphonate (1.5 mmol) in acetonitrile was heated under reflux for 72 h. After cooling, the solvent was extracted under reduced pressure. Yield: 80%.

4.2.1. Ammonium **1a**: 3-[3-(diethoxy-phosphoryl)-propyl]-2-methyl-benzothiazol-3-ium bromide

Compound **1a** was obtained as violet liquid characterized by their NMR parameters.

^{31}P {^{1}H}: δ: 31.4 (CD_3CN).

^{1}H NMR (250 MHz, CD_3CN), δ_{ppm}, J_{Hz}: 7.82–7.87 (m, 2H, H aromatic); 7.26–7.43 (m, 2H, H aromatic); 3.95 (qd, 4H, $J_{HH} = 7.0$, $J_{HP} = 1.2$, O–CH_2); 3.47 (td, 2H, $J_{HH} = 6.5$, $J_{HP} = 0.9$, N–CH_2); 2.72 (s, 3H, CH_3); 2.0 (m, 2H, CH_2); 1.8 (m, 2H, P–CH_2); 1.22 (t, 6H, $J_{HH} = 7.0$).

^{13}C NMR (75.47 MHz, CD_3CN), δ_{ppm}, J_{Hz}: 166.9 (s, C=N); 153.6 (s, Cq aromatic–N); 135.8 (s, Cq aromatic–S); 126.4, 125.9, 122.2, 121.2 (4s, 4 CH aromatic); 61.4 (d, $J_{CP} = 6.4$, O–CH_2); 34.2 (d, $J_{CP} = 18.8$, N–CH_2); 26.2 (d, $J_{CP} = 4.2$, CH_2–*CH_2*); 23.9 (d, $J_{CP} = 142$, P–CH_2); 19.5 (s, CH_3); 16.0 (s, CH_2–*CH_3*).

Mass spectra: $M^+ = 328$ (ESI, positive mode).

4.2.2. Ammonium **2a**: 1-[3-(diethoxy-phosphoryl)-propyl]-2,3,3-trimethyl-3H-indolium bromide

4.2.2.1. Characteristics of **2a**

^{31}P {^{1}H}: δ: 31.0 (CD_3CN).

^{1}H NMR (250 MHz, CD_3CN), δ_{ppm}, J_{Hz}: 7.75–7.14 (m, 4H, H aromatic); 4.0 (qd, 4H, $J_{HH} = 7.0$, $J_{HP} = 1.8$, O–CH_2); 3.5 (td, 2H, $J_{HH} = 6.5$, $J_{HP} = 1.2$, N–CH_2); 2.8 (s, 3H, CH_3); 2.2–1.7 (m, 4H, CH_2 and P–CH_2); 1.7 (s, 6H, $(CH_3)_2$); 1.3 (t, 6H, $J_{HH} = 7.0$, CH_2–CH_3).

^{13}C NMR (75.47 MHz, CD_3CN), δ_{ppm}, J_{Hz}: 188.9 (s, Cq=N); 152.0 (s, Cq aromatic); 145.9 (s, Cq aromatic); 127.7, 125.4, 121.7, 119.2 (4s, CH aromatic); 61.5 (d, $J_{CP} = 6.0$, O–CH_2); 54.6 (s, *C*$(CH_3)_2$); 34.2 (d, $J_{CP} = 18.9$, N–CH_2); 26.1 (s, CH_2); 23.0 (d, $J_{CP} = 113$, P–CH_2); 21.1 (s, C(*CH_3*)$_2$); 20.8 (s, CH_3); 15.8 (s, CH_2–*CH_3*).

Mass spectra: $M^+ = 338.6$ (ESI, positive mode); $M^- = 81.0$ and 79.0 (Br^-, ESI, negative mode).

4.2.3. Ammonium **3a**: 1-[3-(diethoxy-phosphoryl)-propyl]-2,3,3-trimethyl-3H-indolium-5-sulfonate

4.2.3.1. Characteristics of **3a**

^{31}P {^{1}H}: δ: 35.5 (D_2O).

^{1}H NMR (250 MHz, D_2O), δ_{ppm}, J_{Hz}: 7.85–7.53 (m, 3H, H aromatic); 4.1 (qd, 4H, $J_{HH} = 7.0$, $J_{HP} = 1.5$, O–CH_2); 3.5 (t, 2H, $J_{HH} = 6.5$, N–CH_2); 2.2 (m, 4H, CH_2 and P–CH_2); 1.6 (s, 6H, $(CH_3)_2$); 1.3 (t, 6H, $J_{HH} = 7.0$, CH_2–CH_3).

^{13}C NMR (75.47 MHz, D_2O), δ_{ppm}, J_{Hz}: 152.4 (s, Cq aromatic); 146.0 (s, Cq–SO_3); 140.4 (s, Cq–N); 125.8, 119.6, 115.7 (3s, CH aromatic); 63.4 (d, $J_{CP} = 6.5$, O–CH_2); 54.3 (s, $C(CH_3)_2$); 34.1 (d, $J_{CP} = 20.2$, N–CH_2); 25.2 (d, $J_{CP} = 140$, P–CH_2); 22.1 (s, CH_2); 21.8 (s, $(CH_3)_2$); 20.5 (s, CH_3); 15.6 (s, CH_2–CH_3).

Mass spectra: $M^- = 416.25$ (ESI, negative mode); $M + H^+ = 418.15$, $M + K^+ = 455.9$ (ESI, positive mode).

4.2.4. Transformation of **1a** to **1a′**

To ammonium **1a** (0.53 g, 1.3 mmol) is added a mixture of acetic acid (2 ml) and hydrochloric acid 36% (6 ml); the violet solution becomes rapidly colourless. The solution is heated at reflux under magnetic stirring for 48 h to complete the reaction followed by ^{31}P NMR. After work up and drying under vacuum the ammonium **1a′** is obtained as a white solid. Yield: 70%.

4.2.4.1. Characteristics of **1a′**: 2-methyl-3-(3-phosphono-propyl)-benzothiazol-3-ium bromide

^{31}P {^{1}H}: δ: 30.7 (D_2O).

^{1}H NMR (250 MHz, D_2O), δ_{ppm}, J_{Hz}: 8.15–7.7 (m, 4H, H aromatic); 7.4, 7.2 (s, 2H, OH); 3.7 (t, 2H, $J_{HH} = 7.0$, N–CH_2); 3.2 (s, 3H, CH_3); 2.2–1.8 (m, 4H, CH_2 and P–CH_2).

^{13}C NMR (75.47 MHz, D_2O), δ_{ppm}, J_{Hz}: 129.3, 127.9, 123.4, 117.2 (4s, 4 CH aromatic); 45.5 (d, $J_{CP} = 20.4$, N–CH_2); 25.5 (d, $J_{CP} = 6$, CH_2); 24.0 (d, $J_{CP} = 136$, P–CH_2); 17.0 (s, CH_3).

Mass spectra: $M^+ = 272$ (ESI, positive mode); $M^- = 81.0$ and 79.0 (Br^-, ESI, negative mode).

4.2.5. Ammonium **1b**: (3-[2-(diethoxy-phosphoryl)-ethyl]-2-methyl-benzothiazol-3-ium)

2-Methylbenzothiazole (0.697 g, 4.67×10^{-3} mol) and bromodiethylphosphonate (1.14 g, 4.67×10^{-3} mol) in 5 ml of 1,2 dichlorobenzene were heated at 80 °C for 24 h; a ^{31}P NMR control indicates the end of the reaction. After extraction of the solvent under vacuum, the ammonium **1b** was obtained as a pink red oil.

^{31}P {^{1}H}: δ: 26.5 (CD_3CN).

^{1}H NMR (80 MHz, CD_3CN), δ_{ppm}, J_{Hz}: 7.9–7.2 (m, 4H, H aromatic); 4.1 (q, 4H, $J_{HH} = 7.0$, O–CH_2); 3.6 (q, 2H, $J_{HH} = 9$, N–CH_2); 2.8 (s, 3H, CH_3); 2.4 (m, 2H, P–CH_2); 1.3 (t, 6H, $J_{HH} = 7.0$, CH_2–CH_3).

Mass spectra: $M^+ = 314$ (ESI, positive mode); $M^- = 81.0$ and 79.0 (Br^-, ESI, negative mode).

4.3. Formation of phosphonate **B**

The reaction of methylbenzothiazole and bromodiethylphosphonate in acetonitrile gives the vinyldiethylphosphonate **B** instead of the expected ammonium salt. The phosphonate **B** (analog to the commercial product) is characterized as follows.

^{31}P {^{1}H}: δ: 16.1 (CD_3CN).

^{1}H NMR (80 MHz, CD_3CN), δ_{ppm}, J_{Hz}: 6.4 (m, 1H, CH_2); 5.9 (m, 2H, CH and CH_2); 4.0 (qd, 4H, $J_{HH} = 7.0$, $J_{HP} = 1.8$, O–CH_2); 1.3 (t, 6H, $J_{HH} = 7.0$).

Mass spectra: (CI; NH_3) $(M + H)^+ = 165$ (IE); $(M + NH_4)^+ = 182$.

4.4. Synthesis of cyanines

We describe the experimental process for cyanine **4a**; the other compounds are obtained according to a similar way.

Mixture of ammonium **1a** (1.7 g, 4.18 mmol), triethylorthoformate (2 ml) and pyridine (20 ml) is heated at reflux under magnetic stirring for 2 h. A change of colour is immediately observed (deep purple). After cooling to room temperature, 20 ml diethyl ether is added to precipitate the salt and the mixture is kept cold over a night. Cyanine **4a** (violet powder) is isolated by filtration, washed with diethyl ether and dried under vacuum. Yield: 62%.

4.4.1. Cyanine *4a*: 3-[3-(diethoxy-phosphoryl)-propyl] -2-(3-{3-[3-(diethoxy-phosphoryl)-propyl]-3H-benzothiazol -2-ylidene}-propenyl)-benzothiazol-3-ium bromide

^{31}P {^{1}H}: δ: 31.4 (CD_3CN).

^{1}H NMR (250 MHz, CD_3CN), δ_{ppm}, J_{Hz}: 8.5–8.1 (m, 8H, H aromatic); 7.7 (t, 1H, $J_{HH} = 7.5$, CH (chain)); 7.3 (dd, 2H, $J_{HH} = 7.5$, CH (chain)); 4.7 (t, 4H, $J_{HH} = 5$, N–CH_2); 3.9 (qd, 8H, $J_{HH} = 7$, $J_{HP} = 1.8$, O–CH_2); 2.2 (m, 4H, CH_2); 1.9 (m, 4H, P–CH_2); 1.2 (t, 12H, $J_{HH} = 7$, CH_2–CH3).

^{13}C NMR (75.47 MHz, CD_3CN), δ_{ppm}, J_{Hz}: 174.1 (s, C_1 or C_5, chain); 164.9 (s, C=N); 152.6 (s, Cq_{ar}–N); 149.0 (s, C_3–H, chain); 136.1 (s, Cq_{ar}–S); 128.1, 127.7, 127.2, 125.2 (4s, 4 CH aromatic); 112.6 (C_2–H or C_4–H, chain); 61.6 (d, $J_{CP} = 20.3$, O–CH_2); 59.9 (d, $J_{CP} = 18.8$, N–CH_2); 24.5 (s, CH_2); 21.3 (d, $J_{CP} = 141$, P–CH_2); 15.5 (s, CH_2–CH_3).

UV–vis: (CH_3CN) $\lambda_{abs} = 558$ nm, $\varepsilon = 5066$; λ_{abs} (shoulder) = 526 nm, $\varepsilon = 2300$.

Fluorescence: (CH_3CN) $\lambda_{exc} = 530$ nm, $\lambda_{em} = 580$ nm.

Mass spectra: $M^+ = 665.3$ (ESI, positive mode).

4.4.2. Cyanine *5a*: 1-[3-(diethoxy-phosphoryl)-propyl]-2-(3-{1-[3-(diethoxy-phosphoryl)-propyl]-3,3-dimethyl-1,3-dihydro-indol-2-ylidene}-propenyl)-3,3-dimethyl-3H-indolium bromide

^{31}P {^{1}H}: δ: 31.0 (CD_3CN).

^{1}H NMR (250 MHz, CD_3CN), δ_{ppm}, J_{Hz}: 8.0, 7.5–7.0 (m, 9H, H aromatic and CH chain); 6.7 (d, 2H, $J_{HH} = 12.5$, CH chain); 4.7 (t, 4H, $J_{HH} = 7.5$, N–CH_2); 4.0 (qd, 8H, $J_{HH} = 7$, $J_{HP} = 1.8$, O–CH_2); 2.2–1.8 (m, 8H, CH_2 and P–CH_2); 1.6 (s, 12H, $C(CH_3)_2$); 1.2 (t, 12H, $J_{HH} = 7.5$, CH_2–CH_3).

^{13}C NMR (75.47 MHz), δ_{ppm}, J_{Hz}: 174.4 (s, C_1 or C_5 chain); 151.0 (s, Cq aromatic); 150.5 (s, C_3–H, chain); 142.1 (s, Cq aromatic); 129.8, 128.7, 128.3, 125.3 (4s, 4 CH aromatic); 111.4 (s, C_2 or C_4H, chain); 61.6 (d, $J_{CP} = 25$, O–CH_2); 60.6 (d, $J_{CP} = 15$, N–CH_2); 56.9 (s, $C(CH_3)_2$); 27.4 (s, $(CH_3)_2$); 25.2 (s, CH_2); 21.0 (d, $J_{CP} = 140$, P–CH_2); 15.9 (s, CH_2–CH_3).

Mass spectra: $M^+ = 685.8$ (ESI, positive mode).

4.4.3. Cyanine *6a*: 1-[3-(diethoxy-phosphoryl)-propyl]-2-(3-{1-[3-(diethoxy-phosphoryl)-propyl]-3,3-dimethyl-1,3-dihydro-indol-2-ylidene}-propenyl)-3,3-dimethyl-3H-indolium-5-sulfonate bromide

^{31}P {^{1}H}: δ: 33.8 (D_2O).

^{1}H NMR (250 MHz, D_2O), δ_{ppm}, J_{Hz}: 8.2–7.6 (m, 7H, H aromatic and CH chain); 7.5 (d, 2H, $J_{HH} = 11$, CH chain), 4.6 (t, 4H, N–CH_2); 4.1 (qd, 8H, $J_{HH} = 7.0$, $J_{HP} = 1.5$, O–CH_2); 1.9 (m, 4H, CH_2); 1.6 (m, 4H, P–CH_2); 1.35 (s, 12H, $C(CH_3)_2$); 1.25 (t, 12H, $J_{HH} = 7.0$, CH_2–CH_3).

^{13}C NMR (75.47 MHz, D_2O), δ_{ppm}, J_{Hz}: 154.2 (s, C_1 or C_5 chain); 146.5 (s, Cq–SO_3); 145.4 (s, C_3 chain); 139.8 (s, Cq–N); 128.5, 119.4, 118.9 (3s, CH aromatic); 125.6 (s, C_2 or C_4 chain); 63.6 (d, $J_{CP} = 6.8$, O–CH_2); 61.2 (d, $J_{CP} = 18$, N–CH_2); 54.3 (s, $C(CH_3)_2$); 23.8 (s, CH_2); 21.8 (s, $C(CH_3)_2$); 20.8 (d, $J_{CP} = 14.2$, P–CH_2); 15.7 (s, CH_2–CH_3).

UV–vis: (CH_3CN) $\lambda_{abs} = 559$ nm, $\varepsilon = 2316$; λ_{abs} (shoulder) = 528 nm, $\varepsilon = 1708$.

Fluorescence: (CH_3CN) $\lambda_{exc} = 520$ nm, $\lambda_{em} = 577$ nm.

Mass spectra: $M^- = 843.8$ (ESI, negative mode).

4.4.4. Cyanine *4a′*: 3-(3-phosphono-propyl)-2-{3-[3-(3-phosphono-propyl)-3H-benzothiazol-2-ylidene]-propenyl}-benzothiazol-3-ium bromide

The condensation of **1a′** with triethylorthoformate in pyridine and after treatment with ether gives with good yield cyanine **4a′** (violet powder).

^{31}P {^{1}H}: δ: 24.5 (D_2O).

^{1}H NMR (250 MHz, D_2O), δ_{ppm}, J_{Hz}: 8.7–8.6 (m, 4H, H aromatic); 8.4 (t, 1H, $J_{HH} = 7.5$, CH chain); 8.3 (dd, 2H, $J_{HH} = 7.5$, CH chain); 7.9 (m, 4H, H aromatic); 4.5 (t, 4H, $J_{HH} = 5$, N–CH_2); 2.2 (m, 4H, CH_2); 1.5 (m, 4H, P–CH_2).

^{13}C NMR (75.47 MHz, DMSO-d_6), δ_{ppm}, J_{Hz}: 174.1 (s, C_1 or C_5, chain); 147.3, 145.8, 145.3, 145 (4s, 4 CH aromatic); 140.3 (s, C_3–H, chain); 118.5 (Cq aromatic); 61.2 (d, $J_{CP} = 17.6$, N–CH_2); 25.3 (d, $J_{CP} = 3.5$, CH_2); 24.4 (d, $J_{CP} = 137$, P–CH_2).

Mass spectra: $M^+ = 553.6$ (ESI, positive mode).

References

[1] Mazières MR, Romanenko VD, Gudima AO, Payrastre C, Sanchez M, Wolf JG. New diaza pentadienylium salts (cyanine dyes) derived from *N*-silylated phosphinimines and guanidines. Tetrahedron 1995;51: 1405–14.

[2] Rivière F, Romanenko VD, Mazières MR, Sanchez M, Wolf JG. The synthesis of pentadienylium salts via reactions of (5-ethoxy-1,5-diaryl-2,4-pentadienylidene)ethyloxonium perchlorate with hydrazines. Tetrahedron Lett 1996;37:6717–20.

[3] Voitenko Z, Mazières MR, Sanchez M, Wolf JG. Newly substituted pentamethine merocyanines. Part 1: synthesis, physical properties, and synthetic applications. Tetrahedron 2001;57:1059–66.

[4] Viteva L, Gospodova T, Stefanovsky Y, Petrova K, Timtcheva I, Mazières MR, et al. Organometallics in cyanine chemistry – synthesis, reactivity and photophysical properties of some heptamethine merocyanine dyes. Eur J Org Chem 2004:385–94.

[5] Smith JO, Olson DA, Armitage BA. Molecular recognition of PNA-containing hybrids: spontaneous assembly of helical cyanine dye aggregates

on PNA templates. J Am Chem Soc 1999;121:2686–95; Seifert JL, Connor RE, Kushon SA, Wang M, Armitage BA. Spontaneous assembly of helical cyanine dye aggregates on DNA nanotemplates. J Am Chem Soc 1999;121:2987–95.

[6] Griffiths J. Advances in biomedical applications of dyes. In: Colorchem ′04, 10th international conference on dyes, pigments and functional dyes; 2004. p. L22.

[7] Mazières MR, Wetz F, Bellan J, Wolf JG. New phosphorus podands labelled by a cyanine probe: synthesis and fluorescence enhancement with cation complexation. Dyes Pigments 2003;56: 231–8.

[8] Tyutyulkov N, Fabian J, Melhorn A, Dietz F, Tadjer A. Polymethine dyes: structure and properties. Sofia: St Kliment Ohridski University Press; 1991 and references cited therein.

[9] Mishra A, Behera RK, Behera PK, Mishra BK, Behera GB. Cyanines during the 1990s: a review. Chem Rev 2000;100:1973–2011 and references cited therein.

[10] Gruber M, Wetzl B, Oswald B, Enderlein J, Wolfbeis OS. A new fluorescence resonance energy transfer pair and its application to oligonucleotide labeling and fluorescence resonance energy transfer hybridization studies. J Fluoresc 2005;15:207–14.

[11] Boto REF, Oliveira AS, Vieira Ferreira LF, Almeida P. A study of *N*,*N*′-dicarboxyalkyl thiacarbocyanines as cyanine direactive dyes covalently bound to cellulose. Dyes Pigments 2001;48:71–84.

[12] Kudzin ZH, Kotynski A. Synthesis of *O*,*O*-dialkyl 1-aminoalkanephosphonates. Synthesis 1980:1028–32.

[13] Illy H, Funderburk L. Fischer indole synthesis. Direction of cyclization of isopropylmethyl ketone phenylhydrazone. J Org Chem 1968;33:4283–5.