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Oxidation of cationic 1,4-dihydropyridine derivatives as model compounds for putative gene delivery agents

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ABSTRACT

A new synthetic approach to cationic pyridine derivatives is described here. Two different strategies for the synthesis of 1,1′-{[3,5-bis(ethoxycarbonyl)-4-phenylpyridine-2,6-diyl]dimethylene}bispyridinium salts have been developed. The key step of the first strategy relies on electrochemical and chemical oxidation of cationic 1,4-dihydropyridines; the second one involves nucleophilic substitution of pyridine dibromo derivatives.

1. Introduction

1,4-Dihydropyridine (1,4-DHP) derivatives are a group of compounds that play an important role in synthetic, medicinal and bioorganic chemistry.[1] Derivatives of 1,4-dihydropyridines are well-known as calcium channel modulators for the treatment of cardiovascular disorders.[2–4] It is worth underlining that, the 1,4-DHP nucleus is a privileged structure or scaffold that can interact when appropriately decorated with substituents, at diverse receptors and ion channels.[5] 1,4-DHP derivatives possess a broad range of other biological activities, such as antioxidant,[6] anti-inflammatory,[7] radioprotective,[8] anticancer,[9] antidiabetic,[10] immunomodulatory,[11] neuroprotective,[12] antibacterial,[13] antiviral,[14] and reversal of multidrug resistance.[15] However, 1,4-DHPs with these activities might possess also Ca^{2+} regulating activity, which can be considered as a serious side effect and could cause problems in drug development and promotion in the future. Modern strategies for the synthesis of pharmacologically active 1,4-DHPs include minimisation of their Ca^{2+} antagonistic activity.[16] Investigations of the various dihydropyridine derivatives as a novel carrier for specific delivery of drugs to the brain, where 1,4-DHP crosses blood–brain barrier, and oxidises there to quaternary pyridinium salts were also described.[17] During the last decade new and efficient gene delivery systems based on cationic self-assembling amphiphilic 1,4-dihydropyridine derivatives were investigated and elaborated.[18–20]

The oxidation of 1,4-dihydropyridines to their corresponding pyridine derivatives is the most typical and general reaction for this heterocyclic system. One of the main metabolic pathways of biologically active 1,4-dihydropyridine derivatives is the oxidation to their corresponding pyridines, with Cytochrome P450 (CYP)[21,22] as catalyst. Several groups of scientists have developed various methods for aromatisation of 1,4-DHPs and discussed the mechanism of oxidation.[23,24] New aspects of aromatisation of 1,4-DHPs, both electrochemical[25,26] and chemical, have been widely studied. First, for the chemical oxidation of 1,4-dihydropyridines, nitric acid[27] and in situ generated nitric oxide[28,29] were used as powerful oxidising agents. Numerous new inorganic reagents and procedures have been developed for this purpose, for example I_2,[30] solid acids including Oxone®, HIO_3, HIO_6 and polystyrenesulfonic acid,[31] different chromium compounds,[32,33] $KMnO_4$,[34] $Zr(NO_3)_4$,[35] $Bi(NO_3)_3$,[36] $Mn(OAc)_3$,[37] $Pb(OAc)_4$,[38] $RuCl_3$,[39] $Fe(ClO_4)_3$/AcOH[40] etc. Use of microwave-assisted oxidation of 1,4-DHPs was also reported.[41] Furthermore, Hantzsch 1,4-dihydropyridine is widely used as a safe, easy to handle and environmentally benign reagent for the reduction of organic functional groups.[42]

2. Results and discussion

Among the wide range of studies on aromatisation of 1,4-DHP derivatives, there is a lack of data about oxidation of cationic 1,4-dihydropyridines. The elaboration for the synthesis of cationic

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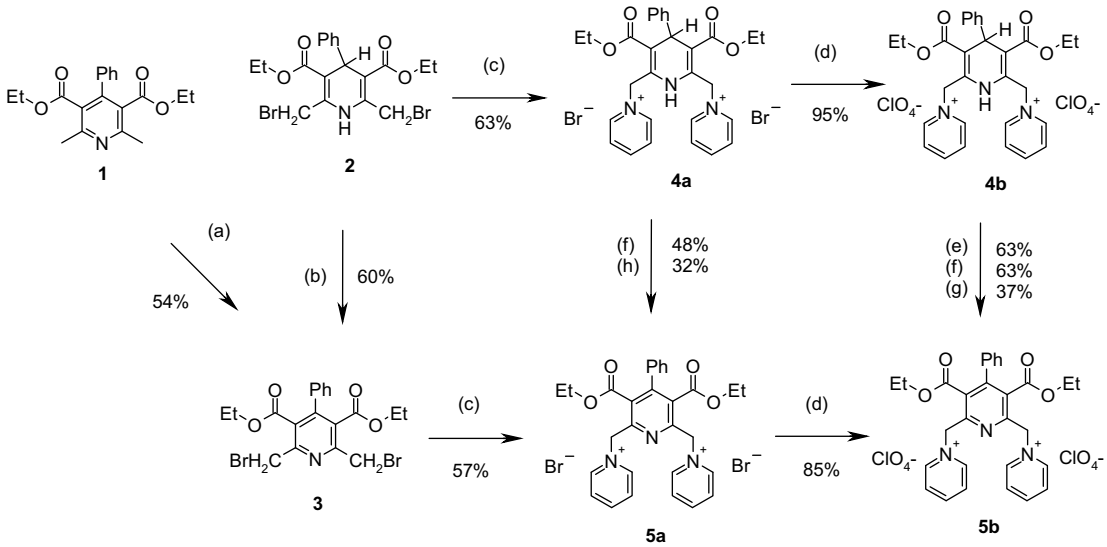
pyridines from 4-phenyl substituted Hantzsch 1,4-dihydropyridines is indispensable for further investigations of the possible metabolic pathways of cationic 1,4-dihydropyridine derivatives and clarification of putative structure–activity relationship. The synthesis of first examples of cationic 1,4-dihydropyridine derivatives with short appendages in the positions 3 and 5 has been performed. Our synthetic plan is shown in Scheme 1.



Scheme 1. Two strategies for the synthesis of cationic pyridine derivatives. Reagents and conditions: (a) Br_2/AcOH at 50 °C, 5 h; (b) $NaNO_2$/AcOH at 50 °C, then at rt, 1.5 h; (c) pyridine in dry acetone, at rt, 3 h; (d) 57% $HClO_4$; hot EtOH, then at rt, 3 h; (e) anodic oxidation in MeCN/0.1 M $NaClO_4$; (f) NBS, MeOH, at 70 °C, 10 h; (g) 10% Pd/C, EtOAc/MeCN at 80 °C, 60 h; (h) SeO_2, AcOH at 50 °C, 5 h.

The compound **1** was obtained according to the classical method.[43] Previously we have reported that the methyl groups of 4-phenyl-3,5-diethoxycarbonyl-2,6-dimethyl-1,4-dihydropyridine[43] were brominated with NBS in methanol at rt to form dibromo derivative **2**.[44] This contrasts sharply with recent report where NBS at rt in 5 min oxidised 4-phenyl substituted Hantzsch 1,4-dihydropyridines to the corresponding pyridines.[45]

Recently, we have reported electrochemical oxidation of cationic 1,4-DHPs.[46] Now we report the first example of chemical oxidation of cationic 1,4-DHP as model compound for putative gene delivery agents (Scheme 1).

There are two strategies to these cationic pyridines:

1. Oxidation of cationic 1,4-DHPs **4a**,**b**:
   a) Direct chemical oxidation of cationic 1,4-DHPs **4a**,**b**;
   b) Direct electrochemical oxidation of cationic 1,4-DHP **4b**.
2. Nucleophilic substitution of compound **3**, which can be obtained via bromination of 4-phenyl-3,5-dialkoxycarbonyl-2,6-dimethylpyridine **1** or oxidation of the dibromo derivative **2**.

We have examined various oxidants for oxidation of 4-phenyl-3,5-dialkoxycarbonyl-2,6-dimethyl-1,4-dihydropyridine cationic derivatives **4a**,**b**. Our initial attempts to oxidise cationic 1,4-DHP derivative **4a** with in situ generated nitric oxide[47] were unsuccessful and led only to destruction of compound **4a**, according to NMR data. Unsuccessful experiments with classical strong oxidising reagents led us to investigate other possibilities of oxidation of cationic 1,4-dihydropyridine derivatives with various reagents previously used for oxidation of neutral 1,4-DHPs, such as tetrachloro-*p*-benzoquinone (*p*-chloranil),[48] 10% Pd/C,[49] and SeO_2.[50] NBS was also tested as oxidising agent as it was previously employed for oxidation of several heterocyclic systems.[51] Some successful examples of oxidation of **4a**,**b** are given in Table 1.

Experimental studies of a variety of reaction conditions, for instance, the amount of oxidising agent, the solvent, reaction temperature and time, showed that the best found reaction conditions for conversion of cationic 1,4-dihydropyridine bromide **4a** or perchlorate **4b** to the corresponding cationic pyridine **5a** or **5b** in reasonable yields were observed when NBS in MeOH was used (entries 1 and 2). Cationic pyridines **5a** and **5b** were accessible in 48% and 63% isolated yields, respectively (Scheme 1). Complete oxidation[49] of neutral 4-phenyl substituted Hantzsch 1,4-DHP with 20 wt% of 10% Pd/C in AcOH occurred at 80 °C only in 2 h. Oxidation of cationic 1,4-dihydropyridine bromide **4a** under similar conditions gave only 23% of conversion to pyridine **5a** in 21 h. Full conversion of cationic 1,4-dihydropyridine perchlorate **4b** into cationic pyridine **5b** was also achieved with 100 wt% of 10% Pd/C (entry 4) with isolated yield of 32% (Scheme1), while in the case of corresponding bromide **4a** at the same conditions only 50% of 1,4-dihydropyridine was oxidised (entry 3). It was possible to reach complete oxidation of 4-phenyl substituted neutral Hantzsch 1,4-DHP with only 1 equiv of SeO_2 at rt in less than

Table 1
Studies of oxidation of cationic 1,4-dihydropyridines **4a**,**b**

Entry	Compound	Oxidant	Solvent	Time, h	Temp, °C	Ratio (DHP:Py), %[a]
1	**4a**	2.6 equiv NBS	MeOH	10	70	0:100
2	**4b**	2.6 equiv NBS	MeOH	10	70	0:100
3	**4a**	100 wt% of 10% Pd/C	EtOAc/MeCN (3:1)	60	80	50:50
4	**4b**	100 wt% of 10% Pd/C	EtOAc/MeCN (3:1)	60	80	0:100
5	**4a**	4 equiv SeO_2	AcOH	5	50	0:100
6	**4b**	4 equiv SeO_2	AcOH/EtOH (5:1)	5	50	70:30
7	**4a**	1 equiv *p*-Chloranil	THF/EtOH (1:1)	6	60	75:25
8	**4b**	1 equiv *p*-Chloranil	THF/EtOH (1:1)	6	60	95:5

[a] The ratio was determined by HPLC and by ^{1}H NMR spectra.

1 h.[50] Oxidation of the cationic 1,4-dihydropyridine bromide **4a** under similar conditions did not occur and only with heating at 50 °C for 3 h oxidation proceeded to approx. 30%. Complete conversion of **4a** into pyridine **5a** was obtained with 4 equiv SeO_2 (entry 5); however, the conversion of perchlorate **4b** was only partial (entry 6), most likely due to the change of solvent from acetic acid to ethanol/acetic acid mixture to improve solubility of **4b**. It has been observed previously that the use of ethanol as a solvent was ineffective for oxidation of Hantzsch 1,4-dihydropyridines using stoichiometric selenium dioxide at ambient temperature.[50] In our hands it was possible to isolate only reaction products containing residual elemental Se. Oxidation of the cationic 1,4-dihydropyridine salts with *p*-chloranil was partial and proceeded to 25% for bromide **4a** (entry 7) and only to 5% for perchlorate **4b** (entry 8). Prolongation of reaction time did not lead to a higher yield of pyridine.

The second synthetic approach to these cationic pyridine derivatives involves as the key step nucleophilic substitution of bromine of pyridine **3**. We have elaborated rather efficient oxidation of the dibromo derivative **2** to compound **3** using sodium nitrite in acetic acid (5 min, at 50 °C) with isolated yield of 60% (Scheme 1). At the temperatures above 50 °C dibromo derivative **2** undergoes lactonisation to give 8-phenyl-5,8-dihydro-1*H*,3*H*-difuro[3,4-*b*:3′,4′-*e*]pyridine-1,7(4*H*)-dione.[44] 4-Substituted pyridine dibromo derivative **3** was also obtained from diethyl 2,6-dimethyl-4-phenyl-pyridine-3,5-dicarboxylate **1** via bromination reaction with bromine in acidic media, in 54% yield (Scheme 1). However, in our studies, it was found that **1** was not brominated with NBS or NBS/benzoyl peroxide or 1,3-dibromo-5,5-dimethylhydantoin in methanol. Though, 2,6-methyl groups of the corresponding 4-phenyl-3,5-dialkoxycarbonyl-2,6-dimethyl-1,4-dihydropyridines were brominated with NBS in methanol in good yields.[44] Using HBr/H_2O_2[52] or NBS/MeOH system[53] combined with UV irradiation (254 nm) bromination of the compound **1** does not occur.

Compound **5a** was obtained in the nucleophilic substitution reaction of 4-phenyl-2,6-dibromomethyl-3,5-diethoxycarbonyl-pyridine **3** with pyridine in dry acetone.

Diperchlorate **4b** was obtained from dibromide **4a** according to a modified method previously reported by us.[46] Electrochemical oxidation of **4b** was studied with cyclic voltammetry (CV) and chronoamperometry. In aprotic solvent CV of compound **4b** shows one irreversible oxidation step (Fig. 1). The presence of two strong electron acceptor groups in the molecule shifts the oxidation potential to 1.7 V, i.e., ~1 V more anodically compared to the oxidation of 4-phenyl substituted Hantzsch dihydropyridines.[54–56]

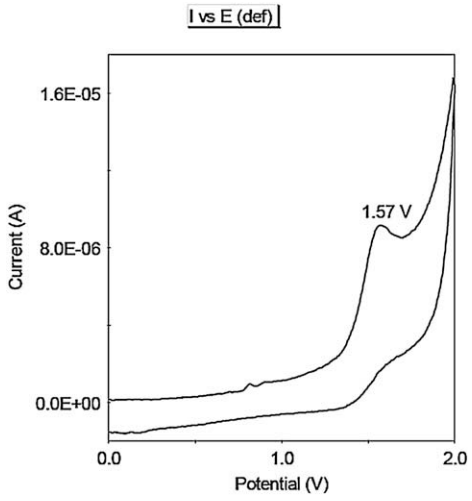


Figure 1. Cyclic voltammogram of **4b** ($c=5\times10^{-4}$ M) recorded on a stationary Pt disk electrode in MeCN/0.1 M $NaClO_4$.

Controlled potential electrolysis of **4b** was performed at 1.70 V in deaerated MeCN. Coulonometric measurements taken during the exhaustive electrolysis indicate that electrochemical oxidation of **4b** is a two-electron process. According to the literature,[57,58] cation radicals, the products of the first electron transfer, are more acidic, compared with the parent neutral 1,4-DHPs. Moreover, the presence of two pyridinium cations in the 2,6-positions of the 1,4-DHP acts in the same way[46] increasing the acidity of N–H bond. Our assumption is that the transfer of the first electron is followed by fast elimination of N–H proton and electrochemical oxidation of **4b** proceeds as the subsequent transfer of electrons and protons, described as ECEC (E—electron transfer; C—proton transfer) mechanism.

For the compound **5b**, monocrystals were obtained, which were characterised by single-crystal X-ray analysis, confirming the NMR data. Figure 2 illustrates the structure of cation **5b**. The calculations for the crystal structure were carried out with the complex of programs,[59,60] and general crystallographic parameters of **5b** are given in Supplementary data. For further details, see crystallographic data for **5b** deposited with the Cambridge Crystallographic Data Centre as Supplementary Publication CCDC 723087.

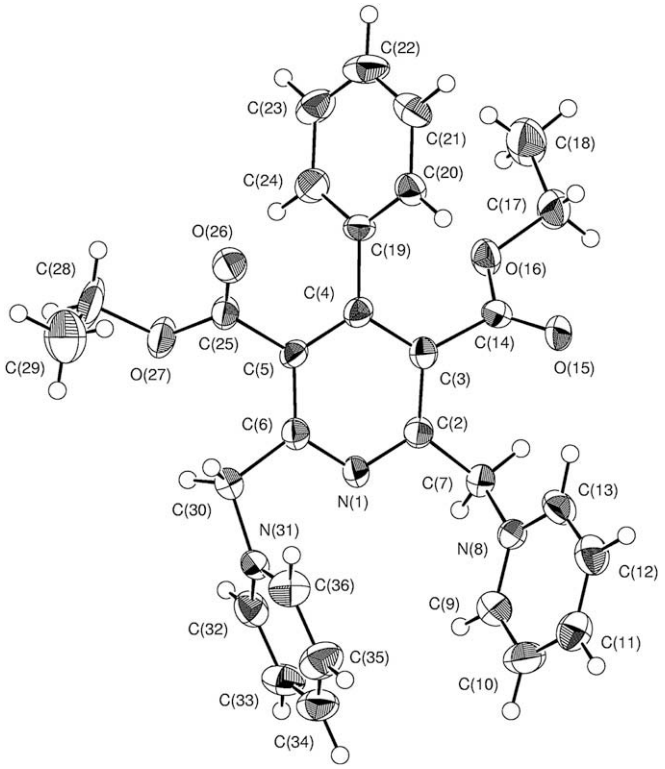


Figure 2. ORTEP representation of structure of cation **5b**.

3. Conclusions

In conclusion, the first chemical oxidation of cationic 1,4-dihydropyridines has been achieved. NBS in MeOH acts as an efficient oxidising agent for conversion of cationic 1,4-dihydropyridine to the corresponding cationic pyridine. Two strategies for the synthesis of cationic pyridine derivatives have been elaborated. Oxidation of cationic 1,4-dihydropyridines with chemical or electrochemical methods leads to cationic pyridines—model compounds for putative gene delivery agents or their metabolites. Our results have shown, that compared with oxidation of neutral 4-phenyl substituted Hantzsch 1,4-dihydropyridine, chemical and electrochemical oxidation is more difficult for cationic 1,4-

dihydropyridines. Alternatively, aromatisation of 1,4-dihydropyridines followed by nucleophilic substitution with pyridine also gives the target cationic pyridine derivatives. Studies of the influence of the cationic substituents on oxidation of 1,4-dihydropyridine ring are currently underway in our laboratory.

4. Experimental

4.1. General

All reagents were purchased from Aldrich, Acros, Fluka or Merck and used without further purification. TLC was performed on 20×20 cm Silica gel TLC-PET F_{254} foils (Fluka). Cyclic voltammetry (CV) and preparative electrolysis were performed on advanced electrochemical system PARSTAT 2273. A three-electrode configuration was used: the working electrode was a stationary Pt disk (d=2 mm), Pt wire served as the counter electrode and an aqueous saturated calomel electrode (SCE) as the reference electrode. Oxidation potential was measured in MeCN/0.1 M $NaClO_4$. Potential scan rate—100 mV/s. Chronoamperometry was carried out in a divided cell with Pt meshes as working and counter electrodes and SCE as a reference electrode. The divided cell was filled with 50 mL MeCN/0.1 M $NaClO_4$ solution and 0.6 g of **4b** was added in the anode compartment. NMR spectra were recorded with a Varian Mercury 200BB (200 MHz) or a Varian 400-MR (400 MHz). Chemical shifts are reported in ppm relative to hexamethyldisiloxane (δ 0.055). Multiplicities are abbreviated as: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. The coupling constants are expressed in Hertz. Diffraction data for **5b** were collected at 173 K on a Bruker-Nonius KappaCCD diffractometer with a low temperature device Oxford Cryostream Plus, using Mo radiation. Powder X-ray diffraction study of **5b** was performed on Rigaku Ultima IV diffractometer. Mass spectral data were determined on an Acquity UPLC system (Waters) connected to a Q-TOF micro hybrid quadrupole time of flight mass spectrometer (Micromass) operating in the ESI positive or negative ion mode on an Acquity UPLC BEH C18 column (1.7 μm, 2.1 mm×50 mm) using a gradient elution with acetonitrile/formic acid (0.1%) in water. The conversions of the reactions were analysed by HPLC on an Alltima CN column, 4.6×150 mm, 5 μm (Alltech) using a LC-1110 pump and a LC-1200 UV/Vis detector at 254 nm (GBC Scientific Equipment). The eluent was acetonitrile/phosphate buffer (pH 2.2; 0.05 M) in water (10:90 by volume) at a flow rate of 1 mL/min. Peak areas were determined electronically with a DP-800 (GBC Scientific Equipment). Melting points were determined on an OptiMelt (SRS Stanford Research Systems). Elemental analyses were determined on an EA 1106 (Carlo Erba Instruments).

4.2. 2,6-Dibromomethyl-4-phenyl-3,5-diethoxycarbonylpyridine (3)

Method A: To a solution of compound **1** (0.19 g, 0.6 mmol) in acetic acid (10 mL), bromine (0.06 mL, 1.2 mmol) was added. The reaction mixture was stirred at 50 °C for 5 h. After cooling to rt, the resulting mixture was diluted with water (20 mL) and extracted with $CHCl_3$ (3×10 mL). The combined organic extracts were dried over $MgSO_4$ and the solvent was removed in vacuo. The residue was crystallised from ethanol giving product **3** as a pale white powder (0.15 g, 54%), mp 80–82 °C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.88 (t, 6H, J=7.1 Hz), 4.01 (q, 4H, J=7.1 Hz), 4.09 (s, 4H), 7.25–7.38 (m, 5H); IR ($CHCl_3$) 1723, 1558 cm^{-1}. MS (+ESI) m/z (relative intensity) 486 ([M+H]$^+$, 55). Anal. Calcd for $C_{19}H_{19}Br_2NO_4$: C, 47.02; H, 3.95; N, 2.89. Found: C, 47.07; H, 3.85; N, 2.89.

Method B: The compound **2** (0.2 g, 0.4 mmol) was dissolved in acetic acid (10 mL) at 50 °C, after which heating was discontinued and sodium nitrite (0.17 g, 2 mmol) was added in several portions. After being stirred at rt for 1.5 h, the resulting mixture was poured into ice water (20 mL) and extracted with $CHCl_3$ (3×10 mL). The combined organic extracts were dried over $MgSO_4$ and the solvent was removed in vacuo. The residue was crystallised from ethanol giving product **3** as a pale white powder (0.12 g, 60%), mp 80–81 °C. ^{1}H NMR ($CDCl_3$, 200 MHz) was identical to that described in Method A for product **3**.

4.3. 1,1′-{[3,5-Bis(ethoxycarbonyl)-4-phenyl-1,4-dihydropyridine-2,6-diyl]dimethylene}-bispyridinium dibromide (4a)

To a solution of compound **2** (0.97 g, 2 mmol) in dry acetone (10 mL), pyridine (0.7 mL, 4 mmol) was added and the reaction mixture was stirred at rt for 3 h. After cooling, the precipitate was filtered off, washed with dry acetone and crystallised from ethanol, giving compound **4a** as a yellow powder (1.33 g, 84%), mp 208–210 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.17 (t, 6H, J=7.0 Hz), 4.07 (q, 4H, J=7.0 Hz), 5.04 (s, 1H), 5.87 and 6.32 (AB-q, 4H, J=13.7 Hz), 7.14–7.27 (m, 5H), 8.13 (dd, 4H, J=6.3 and 7.8 Hz), 8.54 (t, 2H, J=7.8 Hz), 9.30 (d, 4H, J=6.3 Hz), 10.83 (br s, 1H); IR (Nujol) 3573, 3383, 1681, 1628 cm^{-1}; MS (+ESI) m/z (relative intensity) 484 ([M−2Br]$^+$, 4). Anal. Calcd for $C_{29}H_{31}Br_2N_3O_4\cdot H_2O$: C, 52.50; H, 5.01; N, 6.33. Found: C, 52.52; H, 4.86; N, 6.18.

4.4. 1,1′-{[3,5-Bis(ethoxycarbonyl)-4-phenyl-1,4-dihydropyridine-2,6-diyl]dimethylene}-bispyridinium diperchlorate (4b)

To a solution of **4a** (0.36 g, 0.56 mmol) in ethanol (17 mL), perchloric acid (57%, 1.70 mL) was added at reflux temperature, after which the resulting mixture was allowed to cool to rt. The reaction mixture was stirred at rt for 3 h. After cooling, the precipitate was filtered off and crystallised from ethanol to give **4b** as a pale yellow powder (0.11 g, 95%), mp 241–242 °C. ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.08 (t, 6H, J=7.3 Hz), 4.04 (q, 4H, J=7.3 Hz), 5.00 (s, 1H), 5.46 and 6.05 (AB-q, 4H, J=14.6 Hz), 7.26–7.31 (m, 5H), 8.07 (dd, 4H, J=8.1 and 5.9 Hz), 8.56 (t, 2H, J=8.1 Hz), 8.88 (d, 4H, J=5.9 Hz), 9.84 (br s, 1H); IR (Nujol) 3484, 1695, 1634 cm^{-1}; MS (+ESI) m/z (relative intensity) 484 ([M−2ClO$_4$]$^+$, 3). Anal. Calcd for $C_{29}H_{31}Cl_2N_3O_{12}\cdot H_2O$: C, 49.58; H, 4.73; N, 5.98. Found: C, 49.52; H, 4.58; N, 5.91.

4.5. 1,1′-{[3,5-Bis(ethoxycarbonyl)-4-phenylpyridine-2,6-diyl]dimethylene}bispyridinium dibromide (5a)

*4.5.1. Method A: Nucleophilic substitution of **3** with pyridine.* To a solution of compound **3** (0.05 g, 0.15 mmol) in dry acetone (10 mL), pyridine (0.024 mL, 0.3 mmol) was added after which the resulting mixture was stirred at rt for 3 h. After cooling, the precipitate was filtered off, washed with dry acetone and crystallised from ethanol to give **5a** as a white powder (0.04 g, 57%), mp 163 °C (decomp.). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 0.80 (t, 6H, J=6.8 Hz), 4.02 (q, 4H, J=6.8 Hz), 6.14 (s, 4H), 7.17–7.21 (m, 2H), 7.51–7.56 (m, 3H), 8.02 (dd, 4H, J=7.8 and 6.8 Hz), 8.60 (t, 2H, J=7.8 Hz), 8.76 (d, 4H, J=6.8 Hz); IR (Nujol) 1725, 1634 cm^{-1}; MS (+ESI) m/z (relative intensity) 482 ([M−2Br]$^+$, 15). Anal. Calcd for $C_{29}H_{29}Br_2N_3O_4\cdot 3H_2O$: C, 49.94; H, 5.06; N, 6.03. Found: C, 50.16; H, 4.56; N, 5.89.

*4.5.2. Method B: Oxidation of **4a** with NBS.* To a solution of compound **4a** (0.25 g, 0.39 mmol) in methanol (10 mL), NBS (0.18 g, 1 mmol) was added, after which the resulting mixture was stirred at 70 °C for 10 h. After cooling, the solvent was removed in vacuo. The residual crude product was triturated with hexane/ethyl acetate (1:1). The precipitate was filtered off and crystallised from ethanol giving compound **5a** as a white powder

(0.12 g, 48%), mp 163 °C (decomposition). ^{1}H NMR (DMSO-d_6, 200 MHz) was identical to that described for **5a** obtained with method A.

*4.5.3. Method C: Oxidation of **4a** with selenium dioxide.* To a solution of compound **4a** (0.20 g, 0.31 mmol) in acetic acid (10 mL), SeO_2 (0.14 g, 1.26 mmol) was added. The reaction mixture was stirred and heated at 50 °C for 5 h. After cooling to rt, the reaction mixture was quenched with a saturated aqueous $NaHCO_3$ solution and the resulting mixture was extracted with ethyl acetate (3×20 mL). The combined organic extracts were dried over Na_2SO_4 and the solvent was removed in vacuo. The residue was crystallised from ethanol giving a pale orange solid (0.18 g) containing **5a** (40% by HPLC) and residual elemental Se. ^{1}H NMR (DMSO-d_6, 200 MHz) (major peaks) was identical to that described for **5a** obtained with method A: δ 0.81 (t, 6H, J=6.8 Hz), 4.03 (q, 4H, J=6.8 Hz), 6.16 (s, 4H), 7.15–7.24 (m, 2H), 7.49–7.57 (m, 3H), 8.05 (dd, 4H, J=7.8 and 6.8 Hz), 8.57 (t, 2H, J=7.8 Hz), 8.78 (d, 4H, J=6.8 Hz).

4.6. 1,1′-{[3,5-Bis(ethoxycarbonyl)-4-phenylpyridine-2,6-diyl]dimethylene}bispyridinium diperchlorate (5b)

*4.6.1. Method A: Electrochemical oxidation of **4b**.* After exhaustive electrolysis the anolyte was evaporated in vacuo and the residue was stirred with water (10 mL) for 3 h at rt, the precipitate was filtered off and washed with water (10 mL). The precipitate was crystallised from methanol (70 mL) and dried in vacuo, to give 0.38 g (63%) of **5b** as a white powder, mp 235–237 °C; ^{1}H NMR (DMSO-d_6, 200 MHz): δ 0.81 (t, 6H, J=7.0 Hz), 4.06 (q, 4H, J=7.0 Hz), 6.10 (s, 4H), 7.18–7.21 (m, 2H), 7.52–7.55 (m, 3H), 7.99 (dd, 4H, J=5.9 and 8.1 Hz), 8.57 (t, 2H, J=8.1 Hz), 8.72 (d, 4H, J=5.9 Hz); IR (Nujol) 1722, 1624 cm^{-1}; MS (+ESI) m/z (relative intensity) 482 ([M−2ClO$_4$]$^+$, 10). Anal. Calcd for $C_{29}H_{29}Cl_2N_3O_{12}$: C, 51.04; H, 4.28; N, 6.16. Found: C, 50.95; H, 4.15; N, 6.08.

*4.6.2. Method B: Oxidation of **4b** with NBS.* To a solution of compound **4b** (0.25 g, 0.37 mmol) in methanol (10 mL), NBS (0.18 g, 1 mmol) was added, after which the resulting mixture was stirred at 70 °C for 10 h. After cooling, the solvent was removed in vacuo. The residual crude product was triturated with hexane/ethyl acetate (1:1). The precipitate was filtered off and crystallised from ethanol giving compound **5b** as a white powder (0.15 g, 63%), mp 234–236 °C. ^{1}H NMR (DMSO-d_6, 200 MHz) was identical to that described for **5b** obtained with method A.

*4.6.3. Method C: Oxidation of **4b** with Pd/C.* To a solution of the compound **4b** (0.15 g, 0.22 mmol) in a mixture of 30 mL anhydrous ethyl acetate and 10 mL anhydrous MeCN, 10% Pd/C (0.23 g, 0.22 mmol) was added. The mixture was stirred at 80 °C for 60 h. The reaction mixture was filtered twice through Celite and washed with ethyl acetate, dried over Na_2SO_4, filtered through Nylon Filter Membranes (0.2 μm), then the residue was concentrated in vacuo. The residue was crystallised from ethanol giving **5b** as a white powder (0.055 g, 37%), mp 233–236 °C. ^{1}H NMR (DMSO-d_6, 200 MHz) was identical to that described for **5b** obtained with method A. Powder X-ray diffraction data also confirmed this structure.

4.6.4. Method D: Anion exchange. To a solution of **5a** (0.25 g, 0.39 mmol) in ethanol (10 mL), perchloric acid (57%, 1.25 mL) was added at reflux temperature, after which the resulting mixture was allowed to cool to rt. The reaction mixture was stirred at rt for 3 h. After cooling the precipitate was filtered off and crystallised from ethanol to give **5b** as a white powder (0.23 g, 85%), mp 234–236 °C. ^{1}H NMR (DMSO-d_6, 200 MHz) was identical to that described for **5b** obtained with method A.

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Supplementary data

Fluorescence, UV–vis, NMR spectra as well as crystallographic data are available in supplementary data. Supplementary data in the form of a CIF have been deposited with the Cambridge Crystallographic Data Centre for **3** (CCDC 739500). Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2009.08.012.

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