52. Influence of Chelating Groups on the Luminescence Properties of Europium(III) and Terbium(III) Chelates in the 2,2′-Bipyridine Series

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Eight different 2,2′-bipyridine derivatives, *i.e.* **2**, **5**, **8**, **10**, **12**, **13**, **15**, and **19** (*Schemes 1* and *2*), were prepared to study the influence of the chelating groups on the luminescence properties of their Eu^{III} and Tb^{III} chelates. According to our luminescence results, 2,2′-(methylenenitrilo)bis(acetic acid) as well as (methylenenitrilo)bis-(methylphosphonic acid) in 6- and 6′-position of 2,2′-bipyridine is a suitable group when developing luminescent markers for bioaffinity assays based on time-resolved luminescence measurement.

Introduction. – Many aromatic N-compounds can be used for absorbing and transferring energy to lanthanide ions [1]. As a result, these ions enhance intensive luminescence of value in biological applications such as time-resolved luminescence immunoassay. In luminescent lanthanide chelates, 2,2′-bipyridine is one of the most widely studied chromophore. In particular, Eu^{III} and Tb^{III} cryptates, mainly containing three 2,2′-bipyridine moieties as energy-absorbing groups, raised great interest because of their high kinetic stability and their ability to protect lanthanide ions from the influence of the solvent [2]. Eu^{III} and Tb^{III} chelates of substituted 2,2′-bipyridines with a 2,2′-(methylenenitrilo)bis-(acetic acid) group in both the 6- and the 6′-positions are also suitable as luminescent labels [3].

In H_2O solution, 2,2′-bipyridine forms relatively unstable chelates with Eu^{III} and Tb^{III} ions. Additional chelating groups can render the kinetic stability of the chelates sufficiently high, but they may also alter the luminescence properties of the chelates. Furthermore, the net charge of the chelate depends on the number of acidic groups in the ligand and can vary from +3 in cryptates to −5 in certain ligands. The net charge of the chelate can influence on a biological compound to which it is bound, including adsorption properties on different solid phases. Therefore, selection of chelating groups in the ligands must be performed with care to fulfil the needs of the bioaffinity assays based on the luminescence of Eu^{III} and Tb^{III} ions.

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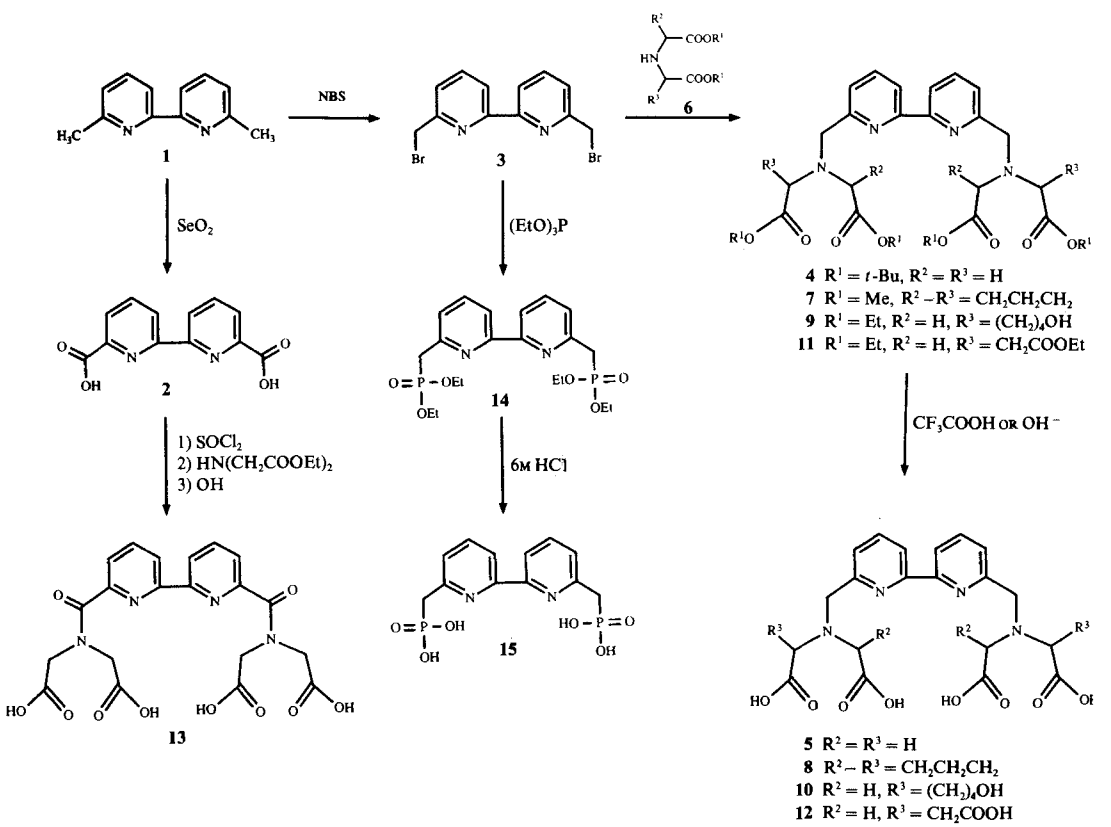
The luminescence properties of Eu^{III} and Tb^{III} chelates depend on many factors, such as on *a*) the triplet energy level of the ligand, *b*) the efficiency of the energy transfer from the ligand to the lanthanide ion, and *c*) the number of H_2O molecules coordinated to the lanthanide ion. The choice of chelating groups coupled to the 2,2′-bipyridine moiety may influence on all these factors.

We synthesized eight different ligands and measured their luminescence properties with Eu^{III} and Tb^{III} ions with the purpose of studying the influence of chelating groups situated symmetrically in the 6- and 6′-positions of 2,2′-bipyridine.

Results and Discussion. – *Syntheses.* The starting material, 6,6′-dimethyl-2,2′-bipyridine (**1**; *Scheme 1*), was prepared using the method described by *Newkome et al.* [4]. The Me groups of **1** can be easily oxidized to carboxylic acids using selenium(IV) oxide in refluxing pyridine [5]. The yield of the resulting 2,2′-bipyridine-6,6′-dicarboxylic acid (**2**) after purification remained quite low (38%). The poor yield was apparently due to inefficient separation rather than to the synthesis itself, since the reaction proceeded quite smoothly (TLC). *Buhleier et al.* synthesized **2** by a more complicated method involving action of CO_2 on 6,6′-dilithio-2,2′-bipyridine [6].

Scheme 1



The synthesis of 6,6′-bis(bromomethyl)-2,2′-bipyridine (**3**) was performed by free-radical bromination using *N*-bromosuccinimide (NBS) [7]. Compound **3** was coupled to di(*tert*-butyl) iminodiacetate (**6**; R^1 = *t*-Bu, $R^2 = R^3 = H$) and the resulting tetra(*tert*-butyl) ester **4** hydrolyzed to tetrakis(acetic acid) **5** using CF_3COOH (see [3]).

Dimethyl *cis*-piperidine-2,6-dicarboxylate (**6**; R^1 = Me, R^2–R^3 = $-(CH_2)_3-$) was prepared by reducing dimethyl pyridine-2,6-dicarboxylate with H_2 using Rh/C as catalyst [8]. Coupling of **6** (R^1 = Me, R^2–R^3 = $-(CH_2)_3-$) to dibromide **3** was then performed analogously to the synthesis of **4**. Base hydrolysis of ester **7** gave ligand **8**.

The 2,2′-{(2,2′-bipyridine-6,6′-diyl)bis{methylene[*N*-(carboxymethyl)nitrilo]}}-6,6′-dihydroxybis(hexanoic acid) (**10**) was prepared by coupling dibromide **3** to diester **6** of suitably modified diethyl iminobis(acetate) and hydrolysis of the intermediate tetraester **9** with base [9].

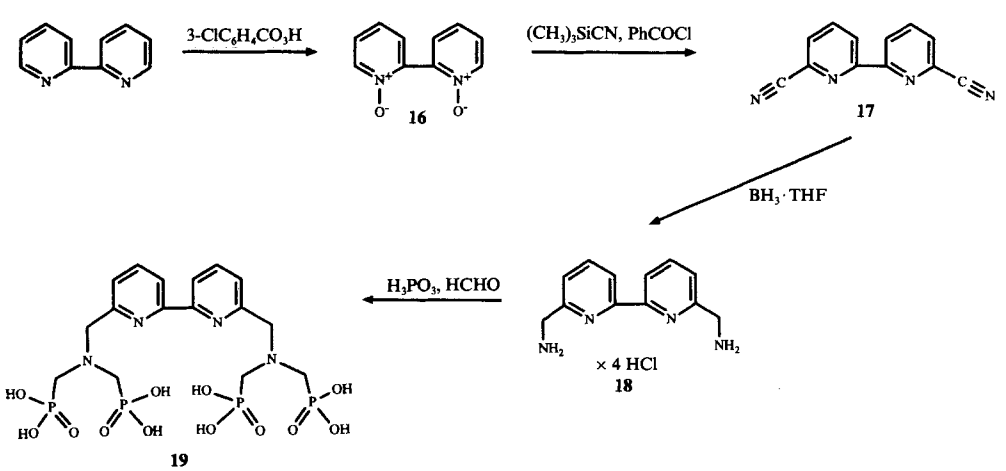
Coupling of ethyl bromoacetate to diethyl DL-2-aminobutane-1,4-dioate [10] yielded diethyl 2-{[(ethoxycarbonyl)methyl]amino}butane-1,4-dioate (**6**; R^1 = Et, R^2 = H, $R^3 = CH_2COOEt$). The further reaction with **3** gave hexaethyl ester **11** and, after hydrolysis with NaOH, hexaacid **12**.

The 2,2′,2″,2‴-[(2,2′-bipyridine-6,6′-diyl)bis(carbonylnitrilo)]tetrakis(acetic acid) (**13**) was prepared by hydrolysis of the corresponding tetraester synthesized from diethyl iminobis(acetate) (**6**; R^1 = Et, $R^2 = R^3$ = H) and **2**.

Newkome et al. synthesized compound **14** using 6,6′-bis(chloromethyl)-2,2′-bipyridine and triethyl phosphite as starting materials [11]. They did not, however, hydrolyze it to the corresponding diphosphonic acid, but instead tried to prepare 6,6′-divinyl-2,2′-bipyridine from it. The hydrolysis of compound **14** to ligand **15** required refluxing in 6M HCl.

Compound **16** (*Scheme 2*), the oxidation product of 2,2′-bipyridine with 3-chloroperbenzoic acid [7], reacted slowly with benzoyl chloride and trimethylsilyl cyanide in a modified *Reissert-Henze* reaction giving 2,2′-bipyridine-6,6′-dicarbonitrile (**17**) as a main product [12]. The latter can also be prepared by nucleophilic substitution of 6,6′-di-

Scheme 2



bromo-2,2′-bipyridine with potassium cyanide [13]. Reduction of the CN groups in **17** was performed with a borane-THF complex [1] [14], and the formed bis(methylamine) **18** reacted further with formaldehyde and phosphorous acid giving **19** [15].

Luminescence. The relative luminescence yields expressed in logarithmic values ($\log R$), excitation maxima (λ_{exc}), and emission decay constants (k_{chel}) of the Eu^{III} and Tb^{III} chelates of the prepared ligands were determined as described in [3]; the results are shown in the *Table*. The decay constants k_{chel} of the chelates were measured only in borate buffer (pH 8.5). Because of the seemingly low variation of the decay constants in D_2O, we used the average of 0.5 ms^{-1} in the study of the H_2O coordination number [16]. Some chelates have two decay constants. The reason might be that the ligands in these cases form chelates with metals in different proportions. This is probable at least with ligands **2** and **15**. Another reason might be competitive energy pathways in the luminescence procedure or isomeric chelate structures [17].

Table. *Relative Luminescence Yields* (as log *R*), *Excitation Maxima* (λ_{exc}), *and Emission Decay Constants* (k_{chel}) *of the Europium(III) and Terbium(III) Chelates of the Prepared Ligands*

Ligand	Chelating group	Eu^{III}			Tb^{III}		
		$\log R$	λ_{exc} [nm]	k_{chel} [ms^{-1}]	$\log R$	λ_{exc} [nm]	k_{chel} [ms^{-1}]
2	COOH (1:1)	5.58	314	1.30, 5.36	4.74	312	2.03, 6.01
5	$CH_2N(CH_2COOH)_2$	5.50	307	1.70	5.27	307	0.82
8	$CH_2NCH(COOH)(CH_2)_3CHCOOH$	5.42	310	1.49	5.23	308	1.04
9	$CH_2N(CH_2COOH)CH(COOH)(CH_2)_4OH$	not measured			5.02	308	0.84
12	$CH_2N(CH_2COOH)CH(COOH)CH_2COOH$	5.30	306	1.45	5.43	306	0.64
13	$C(O)N(CH_2COOH)_2$	too weak			3.43	310	1.36
15	$CH_2PO_3H_2$ (1:1)	4.81	315	3.42, 6.19	4.74	315	2.01
19	$CH_2N(CH_2PO_3H_2)_2$	5.67	318	0.79, 4.67	5.81	314	2.09, 7.63

The excitation wavelengths show very small variations from one compound to another, and they follow closely the absorption spectra of the chelates, which are also all quite similar, with the exception of chelates [Ln^{III}(**2**)] and [Ln^{III}(**13**)]. They have more complex absorption spectra due to the carbonyl groups adjacent to the 2,2′-bipyridine moiety. All ligands form so stable chelates in aqueous solutions that there is a clear shift to a higher wavelength (*ca.* 15–30 nm) in the UV spectra, when the lanthanide chelate is formed. This is caused by the polarization of the 2,2′-bipyridine moiety, its change from s-*trans*- to s-*cis*-conformation and the planarization during the complexation.

Relative luminescence yields are in many cases almost identical for the Tb^{III} and Eu^{III} chelates (except for ligands **2** and **13**). Ligand **13** has very low luminescence both with Eu^{III} and Tb^{III} ions, probably due to the low chelating ability of the amide functions nearby the 2,2′-bipyridine and their influence on the triplet state of the ligand. The use of trimethylene bridges between the acetic-acid groups in ligand **8** make the chelating parts more rigid compared to ligand **5**. This phenomenon is not reflected in the luminescence parameters. The additional carboxylic-acid groups in the chelating part of ligand **12** does not coordinate to the lanthanide ion according to the decay constants. Therefore, the relative luminescence yields for [Ln^{III}(**5**)] and [Ln^{III}(**12**)] are almost identical.

Phosphonate groups cause a shift in the λ_{exc} to longer wavelengths in [Ln^{III}(**15**)] and [Ln^{III}(**19**)] compared to carboxylate-containing compounds. According to the decay constants, the phosphonate groups in both ligands are bidentate. Moreover, especially with [Ln^{III}(**19**)], there are fewer H_2O molecules coordinated to the lanthanide ion than in the corresponding carboxylate chelate. This might also be due to the bulkiness of the phosphonate groups [17]. The somewhat lower log *R* values of [Ln^{III}(**15**)] and higher log *R* values of [Ln^{III}(**19**)] as compared to [Ln^{III}(**5**)] are partly due to the decay times. The relative luminescence yield and decay times of [Eu^{III}(**19**)] are almost the same as those of the Eu^{III} chelate of 2,2′,2″,2‴-[(2,2′:6′,2″-terpyridine-6,6″-diyl)bis(methylenenitrilo)]-tetrakis(acetic acid), whereas [Tb^{III}(**19**)] luminesces somewhat more and has a shorter decay time than the corresponding terbium chelate of 2,2′:6′,2″-terpyridine [1a].

According to our luminescence results, (methylenenitrilo)bis(acetic acid) as well as (methylenenitrilo)bis(methylphosphonic acid) are suitable additional chelating groups for 2,2′-bipyridine when developing luminescent markers for bioaffinity assays.

Experimental Part

General. See [3]. Moreover: IR Spectra: *Perkin-Elmer-1600-FTIR* spectrophotometer; wavenumbers in cm^{-1}.

2,2′-Bipyridine-6,6′-dicarboxylic Acid (**2**). A mixture of 6,6′-dimethyl-2,2′-bipyridine [4] (**1**; 0.50 g, 2.7 mmol), SeO_2 (1.50 g, 13.5 mmol), and pyridine (5.0 ml) was refluxed for 3 h, filtered while still warm and cooled to r.t. The crystallized product was filtered and dissolved in dil. NaOH soln., and the pH was adjusted to 2 with conc. HCl soln. The precipitated product was filtered: 0.25 g (38%). UV (H_2O): 288, 244. UV ([Eu^{III}(**2**)], H_2O): 315, 307, 276, 266, 256. ^{1}H-NMR ((D$_6$)DMSO): 8.15 (*dd*, $J \approx 1.1, 7.6$, 2 H); 8.21 (*t*, $J = 7.6$, 2 H); 8.76 (*dd*, $J = 1.1, 7.6$, 2 H).

Dimethyl cis-*Piperidine-2,6-dicarboxylate* (**6**; R^1 = Me, R^2–R^3 = –$(CH_2)_3$–). A mixture of dimethyl pyridine-2,6-dicarboxylate (2.0 g, 10 mmol), 5% Rh/C (0.25 g), and MeOH (40 ml) was stirred at 50° under H_2 pressure (5.7 MPa) for 20 h. After filtration, the filtrate was evaporated and the product crystallized from MeOH: 0.9 g (45%). M.p. 87° ([18]: 87.6–88.4°). IR (film): 3342 (N–H), 1741 (C=O), 1250 (C–O). ^{1}H-NMR ($CDCl_3$): 1.36–1.57 (*m*, 2 H); 1.97–2.05 (*m*, 4 H); 2.30 (br. *s*, 1 H); 3.37–3.40 (*m*, 2 H); 3.74 (*s*, 6 H).

Tetramethyl 1,1′-[(2,2′-Bipyridine-6,6′-diyl)bis(methylene)]bis(piperidine)-2,2′,6,6′-tetracarboxylate (**7**). As described for **4** [3], **7** was obtained from **6** (R^1 = Me, R^2–R^3 = –$(CH_2)_3$–) and **3**. Yield 81%. UV (EtOH): 289, 239. IR (film): 1732 (C=O), 1577 (C=N), 1198 (C–O). ^{1}H-NMR ($CDCl_3$): 1.79–1.90 (*m*, 12 H); 3.36–3.39 (*m*, 4 H); 3.72 (*s*, 12 H); 4.10 (*s*, 4 H); 7.37 (*d*, $J = 7.8$, 2 H); 7.79 (*t*, $J \approx 7.8$, 2 H); 8.37 (*d*, $J = 7.8$, 2 H).

1,1′-[(2,2′-Bipyridine-6,6′-diyl)bis(methylene)]bis(piperidine)-2,2′,6,6′-tetracarboxylic Acid (**8**). A mixture of **7** (182 mg, 0.326 mmol), acetone (2.0 ml), and 2.5M NaOH (1.0 ml) was stirred at r.t. overnight. Acetone was evaporated, H_2O (0.5 ml) added, and the pH adjusted to 1. The product was filtered: 37 mg (22%). UV (H_2O): 291, 238. UV ([Eu^{III}(**8**)], H_2O): 318 (sh), 307, 245. IR (KBr): 1684 (C=O), 1574 (C=N). ^{1}H-NMR (D_2O): 1.40–1.83 (*m*, 12 H); 3.00–3.15 (*m*, 4 H); 4.02 (*s*, 4 H); 7.29 (*d*, $J \approx 7.8$, 2 H); 7.84 (*t*, $J = 7.8$, 2 H); 8.26 (*d*, $J = 7.8$, 2 H).

Diethyl 2-{[(Ethoxycarbonyl)methyl]amino}butane-1,4-dioate (**6**; R^1 = Et, R^2 = H, R^3 = CH_2COOEt). A mixture of diethyl DL-2-aminobutane-1,4-dioate [10] (1.9 g, 10 mmol), dry K_2CO_3 (3.5 g, 25 mmol), ethyl bromoacetate (1.7 g, 10 mmol), and dry MeCN (80 ml) was stirred for 24 h under N_2. After filtration and evaporation, the oily product was purified by FC (silica gel, petroleum ether/AcOEt 10:1 and 5:3): 2.2 g (81%). IR (film): 3350 (N–H), 1738 (C=O), 1190 (C–O). ^{1}H-NMR ($CDCl_3$): 1.19 (*t*, $J = 7.2$, 3 H); 1.20 (*t*, $J = 7.2$, 3 H); 1.21 (*t*, $J = 7.2$, 3 H); 2.27 (br. *s*, 1 H); 2.63 (*dd*, $J = 6.6, 16.1$, 1 H); 2.70 (*dd*, $J = 5.9, 16.1$, 1 H); 3.37 (*d*, $J = 17.2$, 1 H); 3.45 (*d*, $J = 17.2$, 1 H); 3.5–3.6 (*m*, 1 H); 4.06–4.17 (*m*, 6 H).

Tetraethyl 2,2′-{(2,2′-Bipyridine-6,6′-diyl)bis{methylene{N-[(ethoxycarbonyl)methyl]nitrilo}}}bis(butane-1,4-dioate) (**11**). As described for **4** [3], **11** was obtained from **6** (R^1 = Et, R^2 = H, R^3 = CH_2COOEt) and **3**. The product was purified by FC (silica, petroleum ether/AcOEt 5:1 and 5:2): 27.3 mg (45%). UV (EtOH): 290, 238. IR (film): 1738 (C=O), 1732 (C=O), 1572 (C=N), 1177 (C–O). ^{1}H-NMR ($CDCl_3$): 1.22 (*t*, $J \approx 7.1$, 6 H); 1.23 (*t*,

J = 7.1, 6 H); 1.31 (*t*, J = 7.1, 6 H); 2.77 (*dd*, J = 6.6, 15.7, 2 H); 2.90 (*dd*, J = 8.3, 15.7, 2 H); 3.53 (*d*, J = 17.6, 2 H); 3.68 (*d*, J = 17.6, 2 H); 4.04–4.23 (*m*, 18 H); 7.58 (*d*, J = 7.3, 2 H); 7.77 (*t*, J = 7.3, 3 H); 8.26 (*d*, J = 7.3, 2 H).

2,2'-{(2,2'-Bipyridine-6,6'-diyl)bis{methylene[N-(carboxymethyl)nitrilo]}}bis(butane-1,4-dioic Acid) (**12**). As described for **8**, **12** was obtained from **11**. Yield 43%. UV (H_2O): 288, 237. UV ([Eu^{III}(**12**)], H_2O): 317, 306, 245. IR (KBr): 1572 (C=N). ^{1}H-NMR: the low solubility of **12** in DMSO prevented a proper ^{1}H-NMR analysis; in D_2O, the resolution was very poor.

Diethyl Iminobis(acetate) (**6**; R^1 = Et, R^2 = R^3 = H). $SOCl_2$ (476 g, 4.00 mol) was dropped slowly to cooled EtOH (1200 ml). After stirring at r.t. for 0.5 h, iminobis(acetic acid) (133 g, 1.00 mol) was added and the mixture refluxed overnight. The soln. was evaporated to half the volume, $CHCl_3$ (750 ml) added, and the mixture neutralized with $NaHCO_3$. The org. phase was evaporated and the residue distilled: 149 g (79%). IR (film): 3353 (N–H), 1739 (C=O), 1190 (C–O). ^{1}H-NMR ($CDCl_3$): 1.27 (*t*, J = 7.0, 6 H); 2.03 (*s*, 1 H); 3.43 (*s*, 4 H); 4.17 (*q*, J = 7.0, 4 H).

Tetraethyl 2,2',2'',2'''-[(2,2'-Bipyridine-6,6'-diyl)bis(carbonylnitrilo)]tetrakis(acetate) (**13** with COOEt instead of COOH). A suspension of **2** (0.11 g, 0.39 mmol) and $SOCl_2$ (12.5 ml) was refluxed for 3 h. After evaporation of $SOCl_2$, dry pyridine (15 ml) and **6** (R^1 = Et, R^2 = R^3 = H; 0.19 g, 1.0 mmol) were added. The soln. was stirred at r.t. overnight. Pyridine was evaporated and the product purified by FC (silica gel, $CHCl_3$): 0.11 g (48%). UV (EtOH): 289, 244. ^{1}H-NMR ($CDCl_3$): 1.16 (*t*, J = 7.2, 6 H); 1.33 (*t*, J = 7.2, 6 H); 4.17 (*q*, J = 7.2, 4 H); 4.26 (*q*, J = 7.2, 4 H); 4.44 (*s*, 4 H); 4.50 (*s*, 4 H); 7.90–7.95 (*m*, 4 H); 8.31 (*dd*, J = 2.4, 6.7, 2 H).

2,2',2'',2'''-[(2,2'-Bipyridine-6,6'-diyl)bis(carbonylnitrilo)]tetrakis(acetic Acid) (**13**). The corresponding tetraethyl ester (see above; 54 mg, 0.092 mmol) in acetone (20 ml) and 1M NaOH (4 ml) were stirred for 2 h. Acetone was evaporated and the H_2O soln. neutralized with 1M HCl. The solvent was evaporated. UV (H_2O): 289, 241. UV ([Eu^{III}(**13**)], H_2O): 303, 281, 269, 241. IR (KBr): 1618 (COO^-), 1389 (COO^-). ^{1}H-NMR (D_2O, pD 7): 4.03 (*s*, 4 H); 4.19 (*s*, 4 H); 7.75 (*d*, J = 8, 2 H); 8.14 (*t*, J = 8, 2 H); 8.40 (*d*, J = 8, 2 H).

Tetraethyl 1,1'-(2,2'-Bipyridine-6,6'-diyl)bis(methylphosphonate) (**14**). A soln. of **3** (0.11 g, 0.32 mmol) in $P(OEt)_3$ (5.0 ml) was heated at 150° for 4 h. After evaporation, the product was purified by FC (silica gel, $CHCl_3$ with MeOH gradient). Some cyclohexane was added and the precipitated product filtered: 50 mg (34%). UV (EtOH): 288, 245, 238. ^{1}H-NMR ($CDCl_3$): 1.25 (*t*, J = 7, 12 H); 3.50 (*d*, J = 22, 4 H); 4.04 (*q*, J = 7, 4 H); 4.16 (*q*, J = 7, 4 H); 7.37 (*d*, J = 8, 2 H); 7.76 (*t*, J = 8, 2 H); 8.37 (*d*, J = 8, 2 H).

Disodium Dihydrogen 1,1'-(2,2'-Bipyridine-6,6'-diyl)bis(methylphosphonate) (**15**). A soln. of **14** (20 mg, 0.044 mmol) in 6M HCl (10 ml) was heated at 130° for 16 h. The soln. was evaporated and the residue dissolved in H_2O. After neutralization with Na_2CO_3, the product was precipitated with acetone and dried. UV (H_2O): 292, 235. UV ([Eu^{III}(**15**)], H_2O): 313, 245. ^{1}H-NMR (D_2O, pD 7): 3.15 (*d*, J = 20, 4 H); 7.37–7.57 (*m*, 2 H); 7.70–7.90 (*m*, 4 H).

2,2'-Bipyridine N,N'-Dioxide (**16**). Slowly 3-chloroperbenzoic acid (11.1 g, 64.0 mmol) in $CHCl_3$ (50 ml) was added to 2,2'-bipyridine (4.00 g, 25.6 mmol) and $CHCl_3$ (30 ml). The soln. was stirred overnight. After evaporation, the product was purified by FC (silica gel, $CHCl_3$ with MeOH gradient): 2.72 g (57%). UV (EtOH): 272, 225. IR (film): 1255 (N–O). ^{1}H-NMR ($CDCl_3$): 7.74–7.78 (*m*, 4 H); 7.85 (*t*, J = 8, 2 H); 8.47 (*d*, J = 7, 2 H).

2,2'-Bipyridine-6,6'-dicarbonitrile (**17**). A mixture of **16** (2.72 g, 14.5 mmol), trimethylsilyl cyanide (9.50 g, 95.8 mmol), and CH_2Cl_2 (30 ml) was stirred for 5 min. Benzoyl chloride (8.20 g, 58.3 mmol) was added. After 2 days, 10% K_2CO_3 soln. (100 ml) was added and the mixture extracted with CH_2Cl_2. The org. phase was evaporated, some MeCN added, and the precipitated product filtered: 0.81 g (27%). M.p. 248–251°. UV (EtOH): 297, 289, 246. IR (film): 2237 (C≡N), 1578 (C=N). ^{1}H-NMR ($CDCl_3$): 7.76 (*dd*, J = 2, 8, 2 H); 8.03 (*t*, J = 8, 2 H); 8.73 (*dd*, J = 2, 8, 2 H).

2,2'-Bipyridine-6,6'-bis(methylamine) Tetrahydrochloride (**18**). To a suspension of **17** (0.81 g, 3.9 mmol) in dry THF (25 ml) under N_2, BH_3·THF (1.0M, 50 ml) was added and the mixture stirred overnight. Excessive BH_3 was destroyed by adding MeOH. The solvents were evaporated, and the residue was refluxed for 30 min with EtOH sat. with HCl. The cooled soln. was filtered: 310 mg (22%). UV (H_2O): 286, 236. ^{1}H-NMR (D_2O): 4.46 (*s*, 4 H); 7.55 (*dd*, J = 1, 8, 2 H); 8.06 (*t*, J = 8, 2 H); 8.40 (*dd*, J = 1, 8, 2 H).

1,1',1'',1'''-[(2,2'-Bipyridine-6,6'-diyl)bis(methylenenitrilo)]tetrakis(methylphosphonic Acid) (**19**). H_3PO_3 (120 mg, 1.46 mmol) and 37% formaldehyde soln. (300 µl) was added to a mixture of **18** (80 mg, 0.22 mmol) and 6M HCl (3 ml), and the soln. was refluxed for 3 h. After concentration to 1 ml, the product was precipitated with acetone. The raw product was dissolved in H_2O (1 ml) and $Pb(NO_3)_2$ (70 mg) in H_2O (0.5 ml) added. The precipitated Pb^{II} chelate was filtered and suspended in H_2O (1 ml). H_2S was bubbled through the suspension and the formed PbS filtered off. The filtrate was evaporated. UV (H_2O): 298, 289, 237. UV ([Eu^{III}(**19**)], H_2O): 316, 308, 246. ^{1}H-NMR (D_2O): 3.48 (*d*, J = 12, 8 H); 4.93 (*s*, 4 H); 7.80 (*d*, J = 8, 2 H); 8.28 (*t*, J = 8, 2 H); 8.41 (*d*, J = 8, 2 H).

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