85. Development of Luminescent Europium(III) and Terbium(III) Chelates of 2,2′:6′,2″-Terpyridine Derivatives for Protein Labelling

by **Veli-Matti Mukkala**[1]), **Matti Helenius**[2]), and **Ilkka Hemmilä**

Wallac Oy, P.O. Box 10, SF–20101 Turku

and **Jouko Kankare**

Department of Chemistry, University of Turku, SF–20500 Turku

and **Harri Takalo**[3])*

Centre for Biotechnology, P.O. Box 123, SF–20521 Turku

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The synthesis and luminescence properties are reported for 20 different chelates composed of 2,2′:6′,2″-terpyridine as the energy-absorbing and donating group, Eu^{III} and Tb^{III} as the emitting ions, methylenenitrilo(acetic acids) as the stable chelate-forming moieties, and isothiocyanato or (4,6-dichloro-1,3,5-triazin-2-yl)amino groups as the activated moieties for coupling to biomolecules.

Introduction. – Time-resolved fluorometry combined with long-lifetime emitting lanthanide chelate labels provides an excellent way of creating highly sensitive label technologies for bioaffinity assays [1]. A technology based on dissociative fluorescence enhancement [2], *Delfia*®, has gained wide applications in the field of clinical diagnostics in immunoassays [3] and recently also in DNA hybridization assays [4]. In spite of the high sensitivity obtained, the *Delfia*-type of technology is not suited for all applications, such as fluorescence imaging, immunohistochemistry, or *in situ* hybridization, because after ion dissociation it does not produce spatial information. To use luminescent lanthanide chelates also in *in situ* assays, new chelate labels need to be developed combining all the required features on one ligand, *i.e.* thermodynamic and kinetic chelate stability, hydrophilicity, high absorptivity at a suitable wavelength to facilitate excitation light collection, appropriate triplet state to enable efficient energy transfer and a functional group, allowing the formation of a covalent linkage between the chelate and the target molecule.

The synthesis of functionally optimal stable and highly luminescent chelate labels has long been a challenge for a number of research groups. The results obtained confine to a number of suggested structures found in patent applications. Besides such papers, only a few real applications emerged based on the use of Eu^{III} or Tb^{III} chelates, prepared *e.g.* from 2,2′,2″,2‴-(4′-diazoniophenylethylene-1,2-diimine)tetrakis(acetic acid) [5], monoamide prepared from diethylenetriaminepentaacetic dianhydride and 4-amino-2-

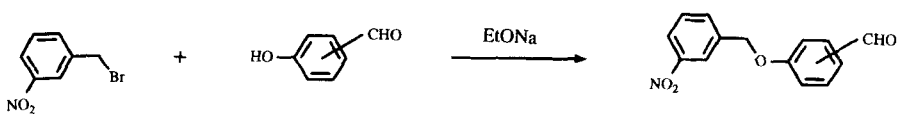
[1]) Present address: also Department of Chemistry, University of Turku, SF–20500 Turku.
[2]) Present address: *Instrumentarium Oy*, SF–20100 Turku.
[3]) Present address: *Wallac Oy*, P.O. Box 10, SF–20101 Turku.

hydroxybenzoic acid [6], 2,2′,2″,2‴-{[4-(4-isothiocyanato-2-methoxyphenyl)pyridine-2,6-diyl]bis(methylenenitrilo)}tetrakis(acetic acid) [7], (bipy.bipy.bipy)-cryptate [8], 2,2′,2″,2‴-{[4-(4′-isothiocyanatophenylethynyl)pyridine-2,6-diyl]bis(methylenenitrilo)}tetrakis(acetic acid) [9], and 2,2′,2″,2‴-{[1-hydroxy-4-(3′-isothiocyanatobenzoyl)benzene-2,6-diyl]bis(methylenenitrilo)}tetrakis(acetic acid) [10]. The chelate labels developed so far, however, do not possess satisfactory properties regarding their luminescence intensity, thermodynamic and kinetic stability, photostability, and suitability as immunolabels.

The Eu^{III} and Tb^{III} chelates of 2,2′:6′,2″-terpyridines and 2,2′-bipyridines were shown to be potential probe alternatives in time-resolved luminometric bioaffinity assays [11] [12]. In this article, we report the synthesis and luminescence properties of 20 different chelates composed of 2,2′:6′,2″-terpyridine as the energy-absorbing and donating group, Eu^{III} and Tb^{III} as the emitting ions, methylenenitrilo(acetic acids) as the stable chelate-forming moieties, and isothiocyanato or (4,6-dichloro-1,3,5-triazin-2-yl)amino groups as the activated moieties for coupling to biomolecules.

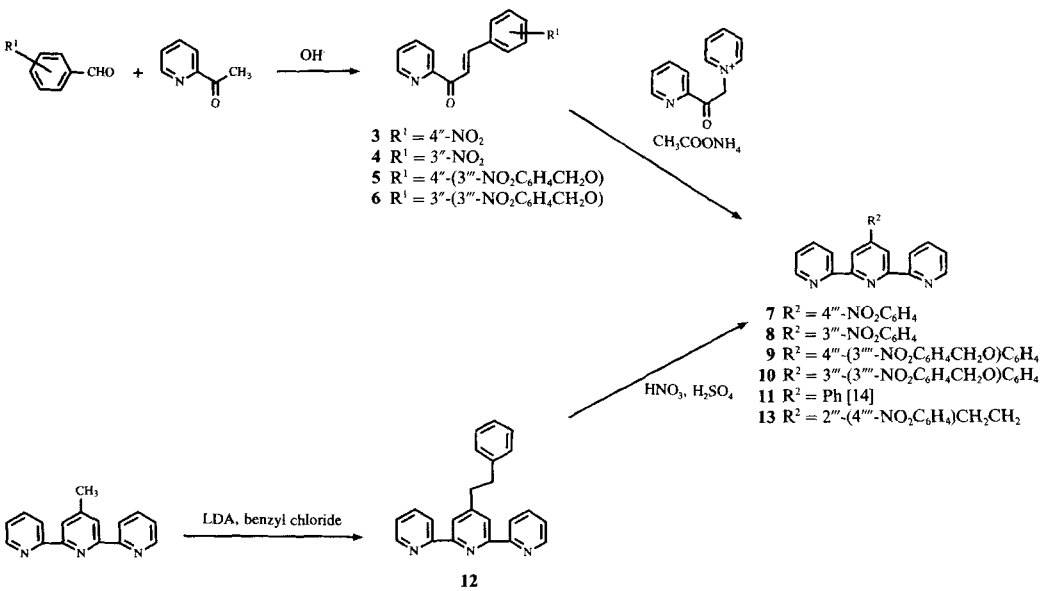
Results and Discussion. – *Syntheses.* The (3′-nitrobenzyloxy)benzaldehydes **1** and **2** were prepared using the *Williamson* ether synthesis by coupling 3-nitrobenzyl bromide to the Na^+ salt of hydroxybenzaldehyde in almost quantitative yields (*Scheme 1*). These and nitrobenzaldehydes were condensed with 2-acetylpyridine by a *Claisen-Schmidt* reaction to (*E*)-prop-2-enones **3**–**6** using KOH or NaOH in a suitable solvent mixture (*Scheme 2*).

Scheme 1



1 *para*-isomer
2 *meta*-isomer

Scheme 2



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Substituted 4′-phenyl-2,2′:6′,2″-terpyridines **7**–**10** were prepared by the reaction of (*E*)-propenones **3**–**6** and *N*-[2-(pyrid-2′-yl)-2-oxoethyl]pyridinium iodide with $AcONH_4$ in AcOH or in MeOH (*Scheme 2*) [13]. According to our results, in MeOH the yield was higher and the amount of colored side products smaller. The 4′-phenyl-2,2′:6′,2″-terpyridine (**11**) was prepared analogously [14]. On alkylation of 4′-methyl-2,2′:6′,2″-terpyridine [15] using BuLi, $(i\text{-}Pr)_2NH$, and benzyl chloride [16] (→ **12**), followed by smooth nitration, 4′-[2‴-(4⁗-nitrophenyl)ethyl]-2,2′:6′,2″-terpyridine (**13**) was obtained.

The terminal pyridine moieties were oxidized with 3-chloroperbenzoic acid to *N*,*N*″-dioxides **14**–**19** (*Scheme 3*) [11] [17]. The modified *Reissert-Henze* reaction [18] yielded 6,6″-dicarbonitriles **20**–**25**, which were reduced with $BH_3 \cdot THF$. Without further purification, the formed bis(aminomethyl) compounds were carboxymethylated with alkyl bromoacetate to tetraesters **26**–**31**. $Et(i\text{-}Pr)_2N$ was preferred to K_2CO_3 as a base because of its better ability to neutralize slightly soluble amine hydrochlorides formed in the reduction of dinitriles. The partial reduction of the NO_2 group of **21** with $BH_3 \cdot THF$ (→ **32**, see below) was quite surprising because the NO_2 group of compounds **22**–**25** tolerated the CN-group reduction.

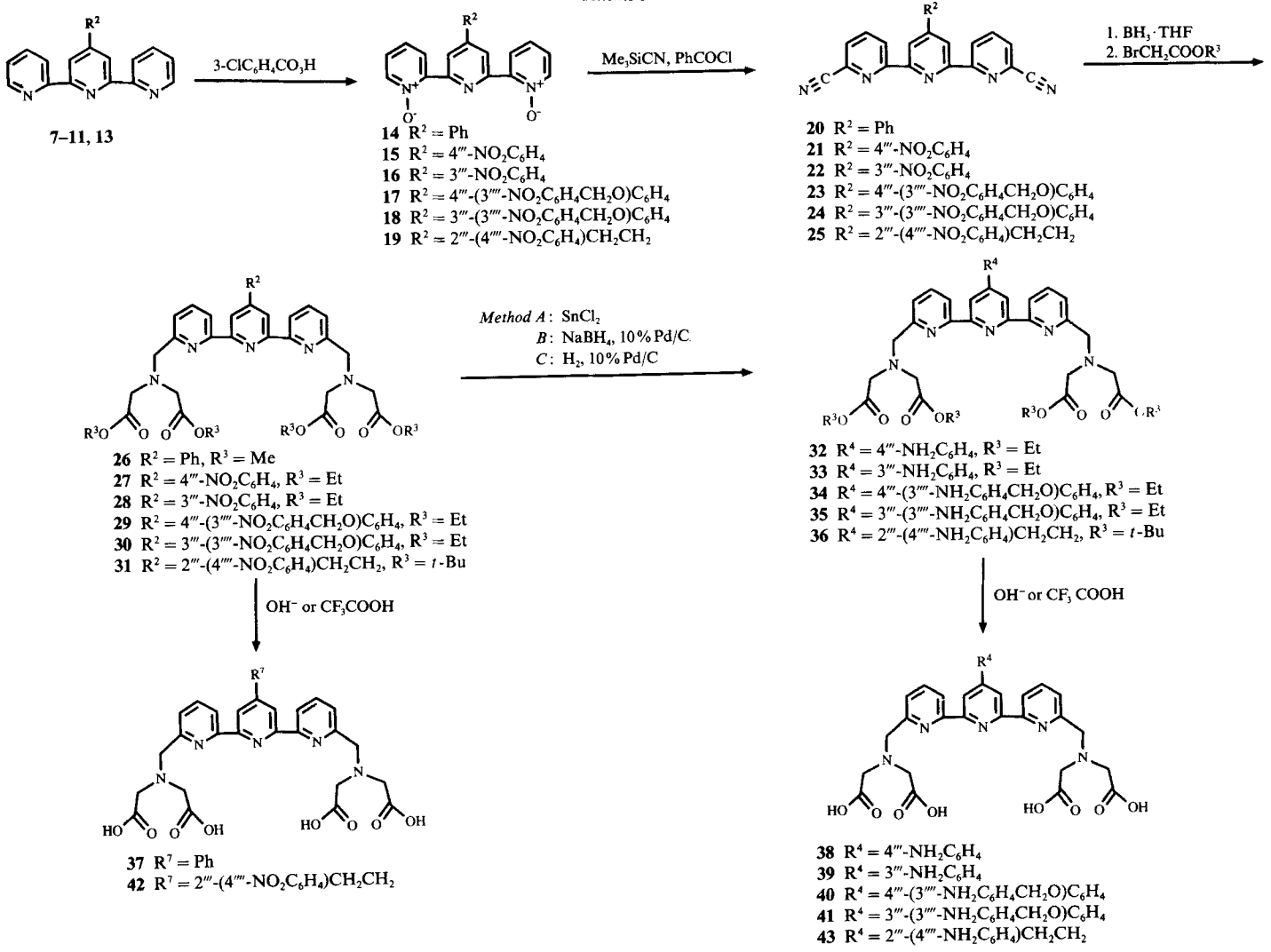
Three different methods were used to reduce the NO_2 group of **27**–**31** (*Scheme 3*). $NaBH_4$ with Pd/C was originally employed [19] [20]. However, the COOEt groups were often partly hydrolyzed under the reaction conditions. Later this method was substituted by the use of $SnCl_2$ in nonacidic abs. EtOH [21]. The Sn ions strongly chelated to the 2,2′:6′,2″-terpyridine moiety were dissociated using diethylenetriaminepentaacetic acid (DTPA) during the purification step; this was shown to give higher yields of **32**–**35** and purer products. Also the benzyloxy groups of **29** and **30** remained unaffected by $SnCl_2$ in abs. EtOH. The conventional use of $SnCl_2$ as reducing agent under acidic conditions did not work. The nitrophenyl group of **31** is isolated from the 2,2′:6′,2″-terpyridine moiety by a stable ethylene bridge; in the presence of this ethylene bridge, the reduction of the NO_2 group was successfully performed with H_2 using Pd/C as a catalyst (→ **36**). The same reduction method applied to **27** gave a rapidly decomposing product. The esters **26** and **31** and the amino-substituted esters **32**–**36** were hydrolyzed with KOH in EtOH or with CF_3COOH (→ **37**–**43**).

Compound **45** was prepared from *N*,*N*′-[2,2′-(pyridine-2,6-diyl)bis(2-oxoethyl)]bis-(pyridinium iodide) (**44**) and sodium 2-oxo-4-phenylbut-3-enoate [22] using the above mentioned *Kröhnke* pyridine synthesis (*Scheme 4*) [13]. Compound **45** was esterified with $MeOH/H_2SO_4$ (→ **46**), and after reduction with $NaBH_4$ in EtOH, diol **47** was transformed with PBr_3 into bis(bromomethyl) derivative **48** without delay, because **47** has a tendency to decompose into an insoluble material. The same phenomenon was also reported by *Newkome et al.* for 9-methyl-1,10-phenanthroline-2-methanol [23]. The coupling of **48** to di(*tert*-butyl) iminobis(acetate) and the hydrolysis of ester **49** to the target tetraacetic acid **50** were performed as usually [11].

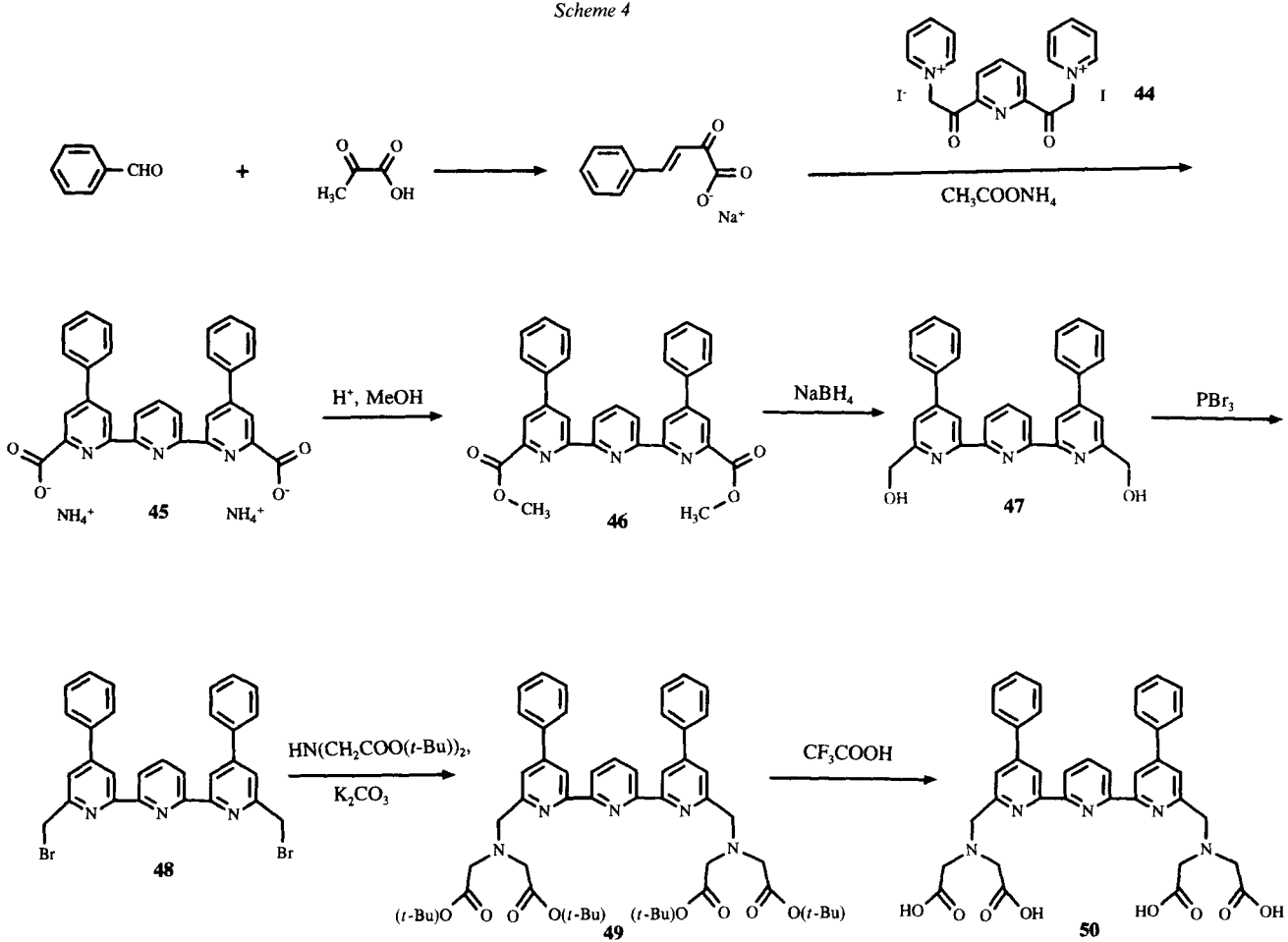
The lanthanide chelates **51**–**66** were prepared by stirring the tetrakis(acetic acids) **37**–**43** and **50** and $LnCl_3$ in slightly acidic solution and precipitating the excess lanthanides as $Ln(OH)_3$ by adjusting the pH to 8.5 (*Scheme 5*). The chelates were precipitated by the addition of acetone.

The activation of the NH_2 group of the chelates by their transformation to the isothiocyanato group (→ **67**–**76**) was performed with thiophosgene in $H_2O/CHCl_3$ by a

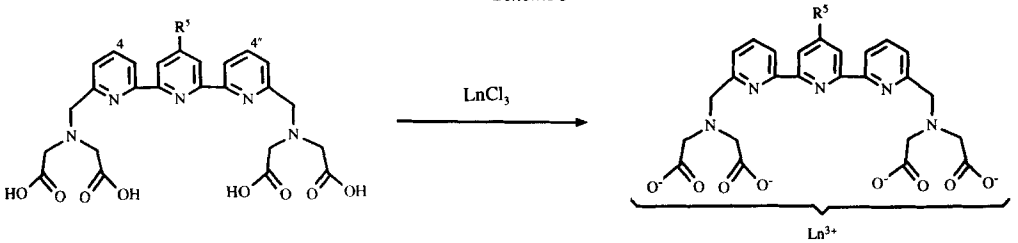
Scheme 3


7–11, 13
3-ClC6H4CO3H
14 R2 = Ph
15 R2 = 4‴-NO2C6H4
16 R2 = 3‴-NO2C6H4
17 R2 = 4‴-(3⁗-NO2C6H4CH2O)C6H4
18 R2 = 3‴-(3⁗-NO2C6H4CH2O)C6H4
19 R2 = 2‴-(4⁗-NO2C6H4)CH2CH2
Me3SiCN, PhCOCl
20 R2 = Ph
21 R2 = 4‴-NO2C6H4
22 R2 = 3‴-NO2C6H4
23 R2 = 4‴-(3⁗-NO2C6H4CH2O)C6H4
24 R2 = 3‴-(3⁗-NO2C6H4CH2O)C6H4
25 R2 = 2‴-(4⁗-NO2C6H4)CH2CH2
1. BH3·THF
2. BrCH2COOR3
26 R2 = Ph, R3 = Me
27 R2 = 4‴-NO2C6H4, R3 = Et
28 R2 = 3‴-NO2C6H4, R3 = Et
29 R2 = 4‴-(3⁗-NO2C6H4CH2O)C6H4, R3 = Et
30 R2 = 3‴-(3⁗-NO2C6H4CH2O)C6H4, R3 = Et
31 R2 = 2‴-(4⁗-NO2C6H4)CH2CH2, R3 = t-Bu
Method A: SnCl2
B: NaBH4, 10% Pd/C
C: H2, 10% Pd/C
32 R4 = 4‴-NH2C6H4, R3 = Et
33 R4 = 3‴-NH2C6H4, R3 = Et
34 R4 = 4‴-(3⁗-NH2C6H4CH2O)C6H4, R3 = Et
35 R4 = 3‴-(3⁗-NH2C6H4CH2O)C6H4, R3 = Et
36 R4 = 2‴-(4⁗-NH2C6H4)CH2CH2, R3 = t-Bu
OH⁻ or CF3COOH
37 R7 = Ph
42 R7 = 2‴-(4⁗-NO2C6H4)CH2CH2
OH⁻ or CF3 COOH
38 R4 = 4‴-NH2C6H4
39 R4 = 3‴-NH2C6H4
40 R4 = 4‴-(3⁗-NH2C6H4CH2O)C6H4
41 R4 = 3‴-(3⁗-NH2C6H4CH2O)C6H4
43 R4 = 2‴-(4⁗-NH2C6H4)CH2CH2

Scheme 4

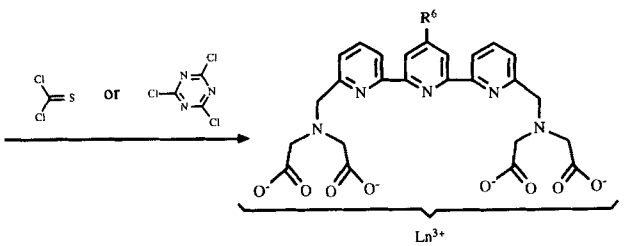

CHO
+
H3C
OH
Na+
I-
I
44
CH3COONH4
NH4+
45
NH4+
H+, MeOH
CH3
46
H3C
NaBH4
OH
47
OH
PBr3
Br
48
Br
HN(CH2COO(t-Bu))2,
K2CO3
(t-Bu)O
O(t-Bu)
(t-Bu)O
O(t-Bu)
49
CF3COOH
HO
OH
HO
OH
50

Scheme 5



37–43 $R^5 = R^4$ or R^7 (see *Scheme 3*)
50 R^5 = H, instead 4-Ph and 4′-Ph

51 R^5 = Ph, Ln = Eu
52 R^5 = Ph, Ln = Tb
53 R^5 = H, instead 4-Ph and 4′-Ph, Ln = Eu
54 R^5 = H, instead 4-Ph and 4′-Ph, Ln = Tb
55 $R^5 = 4'''\text{-}NH_2C_6H_4$, Ln = Eu
56 $R^5 = 4'''\text{-}NH_2C_6H_4$, Ln = Tb
57 $R^5 = 3'''\text{-}NH_2C_6H_4$, Ln = Eu
58 $R^5 = 3'''\text{-}NH_2C_6H_4$, Ln = Tb
59 $R^5 = 4'''\text{-}(3''''\text{-}NH_2C_6H_4CH_2O)C_6H_4$, Ln = Eu
60 $R^5 = 4'''\text{-}(3''''\text{-}NH_2C_6H_4CH_2O)C_6H_4$, Ln = Tb
61 $R^5 = 3'''\text{-}(3''''\text{-}NH_2C_6H_4CH_2O)C_6H_4$, Ln = Eu
62 $R^5 = 3'''\text{-}(3''''\text{-}NH_2C_6H_4CH_2O)C_6H_4$, Ln = Tb
63 $R^5 = 2'''\text{-}(4''''\text{-}NO_2C_6H_4)CH_2CH_2$, Ln = Eu
64 $R^5 = 2'''\text{-}(4''''\text{-}NO_2C_6H_4)CH_2CH_2$, Ln = Tb
65 $R^5 = 2'''\text{-}(4''''\text{-}NH_2C_6H_4)CH_2CH_2$, Ln = Eu
66 $R^5 = 2'''\text{-}(4''''\text{-}NH_2C_6H_4)CH_2CH_2$, Ln = Tb



67 $R^6 = 4'''\text{-}SCNC_6H_4$, Ln = Eu
68 $R^6 = 4'''\text{-}SCNC_6H_4$, Ln = Tb
69 $R^6 = 3'''\text{-}SCNC_6H_4$, Ln = Eu
70 $R^6 = 3'''\text{-}SCNC_6H_4$, Ln = Tb
71 $R^6 = 4'''\text{-}(3''''\text{-}SCNC_6H_4CH_2O)C_6H_4$, Ln = Eu
72 $R^6 = 4'''\text{-}(3''''\text{-}SCNC_6H_4CH_2O)C_6H_4$, Ln = Tb
73 $R^6 = 3'''\text{-}(3''''\text{-}SCNC_6H_4CH_2O)C_6H_4$, Ln = Eu
74 $R^6 = 3'''\text{-}(3''''\text{-}SCNC_6H_4CH_2O)C_6H_4$, Ln = Tb
75 $R^6 = 2'''\text{-}(4''''\text{-}SCNC_6H_4)CH_2CH_2$, Ln = Eu
76 $R^6 = 2'''\text{-}(4''''\text{-}SCNC_6H_4)CH_2CH_2$, Ln = Tb

77 $R^6 = 4'''\text{-}[(Cl_2C_3N_3)NH]C_6H_4$, Ln = Eu
78 $R^6 = 4'''\text{-}[(Cl_2C_3N_3)NH]C_6H_4$, Ln = Tb
79 $R^6 = 3'''\text{-}[(Cl_2C_3N_3)NH]C_6H_4$, Ln = Eu
80 $R^6 = 3'''\text{-}[(Cl_2C_3N_3)NH]C_6H_4$, Ln = Tb
81 $R^6 = 4'''\text{-}\{3''''\text{-}[(Cl_2C_3N_3)NH]C_6H_4CH_2O\}C_6H_4$, Ln = Eu
82 $R^6 = 4'''\text{-}\{3''''\text{-}[(Cl_2C_3N_3)NH]C_6H_4CH_2O\}C_6H_4$, Ln = Tb
83 $R^6 = 3'''\text{-}\{3''''\text{-}[(Cl_2C_3N_3)NH]C_6H_4CH_2O\}C_6H_4$, Ln = Eu
84 $R^6 = 3'''\text{-}\{3''''\text{-}[(Cl_2C_3N_3)NH]C_6H_4CH_2O\}C_6H_4$, Ln = Tb
85 $R^6 = 2'''\text{-}\{4''''\text{-}[(Cl_2C_3N_3)NH]C_6H_4\}CH_2CH_2$, Ln = Eu
86 $R^6 = 2'''\text{-}\{4''''\text{-}[(Cl_2C_3N_3)NH]C_6H_4\}CH_2CH_2$, Ln = Tb

method described previously [24] and the (4,6-dichloro-1,3,5-triazin-2-yl)amino derivatives **77–86** were prepared using 2,4,6-trichloro-1,3,5-triazine.

Luminescence. The excitation maxima (λ_{exc}), luminescence decay times (τ), and luminescence yields ($\varepsilon \cdot \Phi$) for the measured Eu^{III} and Tb^{III} chelates are presented in *Table 1*. The chelates of the unsubstituted 2,2′,2″,2‴-(2,2′:6′,2″-terpyridine-6,6″-diyl)bis(methylenenitrilo)]tetrakis(acetic acid) (see **51**, R^5 = H) and the chelates **51–54** and **63–66** were measured directly, whereas the activated chelates **67–86** were measured after coupling to a protein.

The prepared chelates usually exhibited two excitation maxima with almost equal intensities (see *Fig.*). In *p*-substituted labels, the longer excitation wavelength usually has a little higher intensity, with *m*-isomers the situation is reverse. In *Table 1*, the results are shown only for the strongest excitation peak. However, all chelates can be excited at a wavelength of *ca.* 330 nm, which is a more convenient excitation wavelength for instrumental reasons relating to UV transmission of lenses, filters, cuvettes, and glass slides.

Table 1. *The Excitation Maxima* (λ_{exc}), *Luminescence Decay Times* (τ), *and Luminescence Yields* ($\varepsilon \cdot \Phi$) *of the Europium(III) and Terbium(III) Chelates of 2,2′,2″,2‴-[(2,2′:6′,2″-Terpyridine-6,6″-diyl)bis(methylenenitrilo)]tetrakis(acetic Acid) Derivatives*

	Eu^{3+}			Tb^{3+}		
	λ_{exc} [nm]	λ [μs]	$\varepsilon \cdot \Phi$	λ_{exc} [nm]	τ [μs]	$\varepsilon \cdot \Phi$
Uncoupled chelates						
Parent compound (see **51** and **52** with R^5 = H)[a])	334	1310	2100	333	1100	3800
4′-Phenyl (**51** and **52**)	293	1210	1970	293	530	1900
4,4″-Diphenyl (**53** and **54**)	339	1300	3900	339	260	1500
4′-[2‴-(4⁗-Nitrophenyl)ethyl] (**63** and **64**)	332	1340	580	270	577	32
4′-[2‴-(4⁗-Aminophenyl)ethyl] (**65** and **66**)	331	1240	220	332	697	53
Chelates coupled to proteins						
4′-(4‴-Isothiocyanatophenyl) (**67** and **68**)	340	1400	2100	too low for measurement		
4′-{4‴-[(4,6-Dichloro-1,3,5-triazin-2-yl)amino]phenyl} (**77** and **78**)	340	1590	2600	320	–	9
4′-(3‴-Isothiocyanatophenyl) (**69** and **70**)	295	1540	1300	too low for measurement		
4′-{3‴-[(4,6-Dichloro-1,3,5-triazin-2-yl)amino]phenyl} (**79** and **80**)	330	1500	600	too low for measurement		
4′-[4‴-(3⁗-Isothiocyanatobenzyloxy)phenyl] (**71** and **72**)	340	1520	2500	334	50	190
4′-{4‴-{3⁗-[(4,6-Dichloro-1,3,5-triazin-2-yl)amino]benzyloxy}phenyl} (**81** and **82**)	333	1450	1800	333	110	190
4′-[3‴-(3⁗-Isothiocyanatobenzyloxy)phenyl] (**73** and **74**)	297	1200	790	300	30	35
4′-{3‴-{3⁗-[(4,6-Dichloro-1,3,5-triazin-2-yl)amino]benzyloxy}phenyl} (**83** and **84**)	296	1380	2600	295	62	190
4′-[2‴-(4⁗-Isothiocyanatophenyl)ethyl] (**75** and **76**)	332	1490	680	332	91	130
4′-{2‴-{4⁗-[(4,6-Dichloro-1,3,5-triazin-2-yl)amino]phenyl}ethyl} (**85** and **86**)	332	1490	840	331	450	490

[a]) See [11].

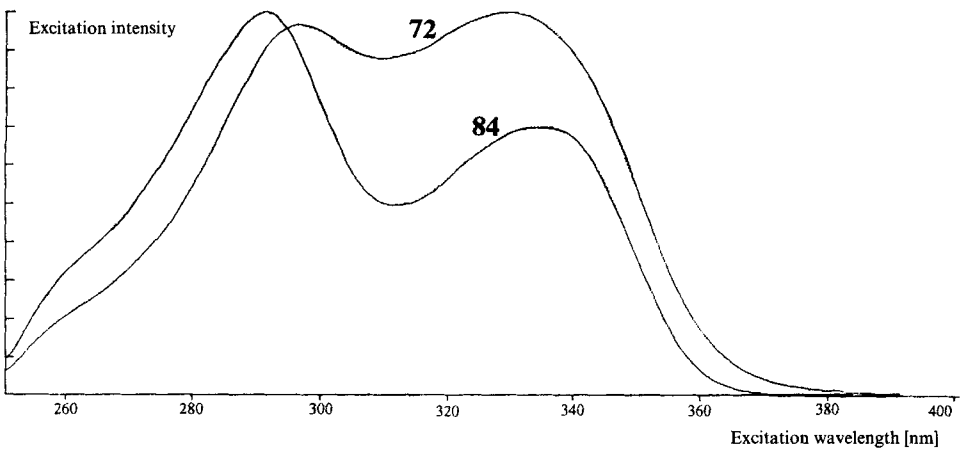


Figure. *The excitation spectra of chelates* **72** *and* **84**

The parent compound showed relatively high luminescence intensities for both Eu^{III} and Tb^{III}. The Ph substituents in the two terminal pyridine rings of chelate **53** increased the total emission, whereas substituents comprising aromatic NO_2 (**63**) or NH_2 groups (**65**) clearly quenched the luminescence of Eu^{III} chelates, even though the groups were not directly attached to excited aromatic moieties. This quenching was, however, partly recovered upon further modification of the NH_2 group into activated isothiocyanato or (4,6-dichloro-1,3,5-triazin-2-yl)amino groups, and upon coupling to a protein. Generally, the activation of the amino-chelates transforms them to luminescent species on thin-layer chromatography (TLC). The Eu^{III} chelates coupled to a protein had luminescence intensities almost identical to those of their parent compounds. The *p*-substituted chelates demonstrated moderately higher luminescence and also more prominent excitation in the 330-nm region than *m*-isomers (with the exception of **83**).

With regard to their decay times, the Eu^{III} chelates behaved quite predictably as nine-dentate chelates. The decay times of Eu^{III} chelates in aqueous buffer were almost identical to all chelates studied. To calculate the exact number of coordinated H_2O molecules, the measurements should be done also in D_2O [25]. However, based on the estimation of an average decay constant of 0.5 ms^{-1} for an Eu^{III} chelate in D_2O, the decay constant obtained in aqueous media indicated that the chelates coupled to the protein contained *ca.* 0.2 coordinated H_2O molecules in the first coordination sphere. The values for uncoupled chelates were somewhat higher, on the average 0.3 coordinated H_2O molecules.

Two different coupling reactions of the chelates with proteins were tested. The reaction of (4,6-dichloro-1,3,5-triazin-2-yl)amino-activated chelates clearly showed to be more efficient enabling high labelling yields under conditions with moderate reagent excess which, however, also implied a risk of overlabelling. As compared to the use of isothiocyanato-activated chelates for coupling, the (dichlorotriazinyl)amino-activated chelates yielded products with good adsorption properties, thus greatly simplifying the chromatographic separation of labelled protein from uncoupled chelates. The coupling method applied had a minor and unpredictable effect on the final luminescence yield.

Of the studied chelates, the parent structure showed the highest luminescence intensity with the Tb^{III} ion (see **52** with R^5 = H). The substituents in the aromatic part seemed to decrease the luminescence yields and also the decay times considerably (*Table 1*). It is supposed that this decrease may be a result of the lowered ligand triplet state causing an energy leakage back to the ligand triplet state [26]. Similarly to Eu^{III} chelates, the NO_2- and NH_2-containing substituents effectively quenched Tb^{III} luminescence, even though this was partly overcome by further modification and coupling. The tested Tb^{III} chelates behaved quite unpredictably upon conjugation to the protein, both with regard to the decay times and luminescence intensities.

The behavior of decay times of Tb^{III} chelates was much more complicated than that of Eu^{III} chelates. In addition to the decreased decay times observed with the more substituted parent compounds **52** and **54**, the protein surface seemed to have a very significant effect on Tb^{III} luminescence. The shortest decay time, 30 μs, was observed with the protein-conjugated chelate **74**. Even the coupling method had some effect on the decay times, and the thiourea-coupled chelates (**72**, **74**, **76**) were more rapidly quenched than the corresponding 6-chloro-1,3,5-triazin-2,4-diyl derivatives (**82**, **84**, **86**). The unexpectedly short decay times strongly decrease the applicability of the chelates in time-resolved luminometric technologies. To rule out the effect of coordinated H_2O molecules on the observed decay times, chelates **72** and **82** were also measured as their condensation products with tris(hydroxymethyl)aminomethane (= 2-amino-2-(hydroxymethyl)propane-1,3-diol; *Tris*) both in H_2O and D_2O [25]. As shown in *Table 2*, the effect of H_2O molecules bound to the chelate is only minor, and the short decay times are rather caused by some other quenching route in addition to OH oscillation. A similar unexpected shortening of Tb^{III} decay times was observed with β-diketone chelates, in which the number of F-atoms attached to the diketone was inversely proportional to the length of the Tb^{III} decay time, but had a minor effect on quantum yield [27]. Unfortunately, the coupling of the chelates to protein further decreased both the quantum yields and decay times.

Table 2. *Effect of H_2O, D_2O, and Protein Surface on Luminescence Yields ($\varepsilon \cdot \Phi$) and Decay Times (τ) of Terbium(III) Chelates* **72** *and* **82**

Chelate	*Tris* conjugate				Protein conjugate	
	$(\varepsilon \cdot \Phi)_{H_2O}$	$(\varepsilon \cdot \Phi)_{D_2O}$	τ_{H_2O}	τ_{D_2O}	$(\varepsilon \cdot \Phi)_{H_2O}$	τ_{H_2O}
72	530	596	275	340	190	50
82	226	398	220	316	190	110

The Tb^{III} reagent with the best labelling properties was (4,6-dichloro-1,3,5-triazin-2-yl)amino-activated phenylethyl derivative **86**, which had a reasonably high luminescence intensity although the decay time was still quite short (450 μs).

According to the present experiments, it is possible to develop highly luminescent Eu^{III} chelates which can be coupled to proteins, *e.g.* antibodies, and used as tracers in immunological binding assays. The chelates are extremely stable both with respect to thermodynamic and kinetic stability enabling their use also under demanding conditions, such as hybridization assays performed at high temperatures in the presence of high excess of

EDTA. For double-label assays, Tb^{III} chelates would be the second choice. Tb^{III} Complexes, however, show some unexpected energy leakage routes making these chelates much more sensitive to the environment and thus giving rise to a decreased quantum yield and shortened decay times. With Tb^{III} chelates, the functional group needed for coupling has to be energetically insulated from the excited part, and it is more difficult to forecast from the parent structures the properties of functionalized chelates coupled to target molecules.

Experimental Part

General. Flash chromatography = FC. M.p.: uncorrected. UV Spectra: *Beckman-DU8* or *Shimadzu-UV-2100* spectrophotometer; λ_{max} in nm. Luminescence spectra: decay times τ in μs and luminescence intensities were measured with a *Perkin-Elmer-LS-5* luminescence spectrometer combined with a *Perkin-Elmer-CLS* data station; high-sensitivity fluorometry of the lanthanides, their quantitations, and the measurement of luminescence intensities from plastic surfaces (microtitration strip wells) were performed with a time-resolved *Wallac-1234-Delfia-Research* fluorometer combined with a PC and *Wallac-MultiCalc* program. IR Spectra: *Perkin-Elmer-1600-FTIR.* ^{1}H-NMR Spectra: 400-MHz *Jeol-GX-400*; tetramethylsilane or sodium 3-(trimethylsilyl)propane-1-sulfonate as internal standards, chemical shifts δ in ppm, coupling constants J in Hz.

1. *Synthesis of* **1** *and* **2**: *General Procedure.* Na (0.46 g, 20 mmol) was added to abs. EtOH (100 ml). After Na had reacted, 4- or 3-hydroxybenzaldehyde (2.44 g, 20 mmol) and 3-nitrobenzyl bromide (4.32 g, 20 mmol) were added, and the mixture was refluxed for 1 h. After evaporation, the residue was dissolved in $CHCl_3$ (100 ml), washed with 1M NaOH (50 ml) and H_2O (50 ml), dried (Na_2SO_4), and evaporated.

4-(3'-Nitrobenzyloxy)benzaldehyde (**1**). Yield: 99%. UV (EtOH): 268. IR (KBr): 1697 (C=O), 1529 (NO_2), 1352 (NO_2), 1267 (C–O–C). ^{1}H-NMR ($CDCl_3$): 5.25 (*s*, 2 H); 7.10 (*d*, *J* = 9, 2 H); 7.61 (*t*, *J* = 8, 1 H); 7.79 (*d*, *J* = 8, 1 H); 7.87 (*d*, *J* = 9, 2 H); 8.22 (*d*, *J* = 8, 1 H); 8.34 (*s*, 1 H); 9.91 (*s*, 1 H).

3-(3'-Nitrobenzyloxy)benzaldehyde (**2**). Yield: 94%. UV (EtOH): 254. IR (KBr): 1693 (C=O), 1525 (NO_2), 1348 (NO_2), 1263 (C–O–C). ^{1}H-NMR ($CDCl_3$): 5.23 (*s*, 1 H); 7.27–7.30 (*m*, 1 H); 7.48–7.54 (*m*, 2 H); 7.52 (*s*, 1 H); 7.60 (*t*, *J* = 8, 1 H); 7.78 (*d*, *J* = 8, 1 H); 8.22 (*d*, *J* = 8, 1 H); 8.35 (*s*, 1 H); 9.99 (*s*, 1 H).

2. *Synthesis of* **3** *and* **4**: *General Procedure.* To an ice-cold soln. of KOH (30.9 g, 0.55 mol) in MeOH (1100 ml) and H_2O (220 ml), **1** or **2** (83.7 g, 0.55 mol) was added. After dissolution, 2-acetylpyridine (67.1 g, 0.55 mol) was added within 10 min, mixture stirred for 3 h in ice-bath and then filtered, and the precipitate washed with cold MeOH.

(E)-3-(4''-Nitrophenyl)-1-(pyrid-2'-yl)prop-2-enone (**3**). The crude product was crystallized from EtOH. Yield: 82%. UV (EtOH): 319. IR (film): 1676 (C=O), 1527 (NO_2), 1349 (NO_2). ^{1}H-NMR ($CDCl_3$): 7.54 (*ddd*, *J* = 1,5, 8, 1 H); 7.87 (*d*, *J* = 9, 2 H); 7.91 (*dt*, *J* = 2, 8, 1 H); 7.92 (*d*, *J* = 16, 1 H); 8.21 (*td*, *J* = 1, 8, 1 H); 8.27 (*d*, *J* = 9, 2 H); 8.43 (*d*, *J* = 16, 1 H); 8.75 (*m*, 1 H).

(E)-3-(3''-Nitrophenyl)-1-(pyrid-2'-yl)prop-2-enone (**4**). Yield: 93%. UV (EtOH): 298. IR (film): 1672 (C=O), 1513 (NO_2), 1343 (NO_2). ^{1}H-NMR ($CDCl_3$): 7.54 (*ddd*, *J* = 1, 5, 8, 1 H); 7.62 (*t*, *J* = 8, 1 H); 7.91 (*dt*, *J* = 1, 8, 1 H); 7.94 (*d*, *J* = 16, 1 H); 8.01 (*d*, *J* = 8, 1 H); 8.21 (*d*, *J* = 8, 1 H); 8.26 (*dd*, *J* = 1, 8, 1 H); 8.44 (*d*, *J* = 16, 1 H); 8.59 (br. *s*, 1 H); 8.78 (br. *d*, *J* = 5, 1 H).

3. *Synthesis of* **5** *and* **6**: *General Procedure.* A soln. of **1** or **2** (4.81 g, 18.7 mmol) in THF (30 ml) was added to a mixture of 2-acetylpyridine (2.27 g, 18.7 mmol), NaOH (0.75 g, 18.7 mmol), EtOH (30 ml), and H_2O (10 ml). After stirring for 5 h at r.t., the mixture was evaporated and the residue triturated with H_2O (150 ml). The solid material was filtered and washed with H_2O and cold EtOH.

(E)-3-[4''-(3'''-Nitrobenzyloxy)phenyl]-1-(pyrid-2'-yl)prop-2-enone (**5**). Yield: 88%. UV (EtOH): 346, 248. IR (KBr): 1663 (C=O), 1537 (NO_2), 1351 (NO_2), 1259 (C–O–C). ^{1}H-NMR ($CDCl_3$): 5.21 (*s*, 2 H); 7.02 (*d*, *J* = 9, 2 H); 7.49 (*ddd*, *J* = 1, 5, 8, 1 H); 7.59 (*t*, *J* = 8, 1 H); 7.72 (*d*, *J* = 9, 2 H); 7.79 (*d*, *J* = 8, 1 H); 7.87 (*dd*, *J* = 1, 8, 1 H); 7.92 (*d*, *J* = 16, 1 H); 8.20 (*t*, *J* = 8, 1 H); 8.21 (*d*, *J* = 16, 1 H); 8.22 (*d*, *J* = 8, 1 H); 8.34 (*s*, 1 H); 8.73–8.76 (*m*, 1 H).

(E)-3-[3''-(3'''-Nitrobenzyloxy)phenyl]-1-(pyrid-2'-yl)prop-2-enone (**6**). Yield: 72%. UV (EtOH): 313, 257. IR (film): 1669 (C=O), 1528 (NO_2), 1352 (NO_2), 1274 (C–O–C). ^{1}H-NMR ($CDCl_3$): 5.22 (*s*, 2 H); 7.03–7.06 (*m*, 1 H); 7.35–7.38 (*m*, 2 H); 7.38 (*s*, 1 H); 7.49–7.53 (*m*, 1 H); 7.60 (*t*, *J* = 8, 1 H); 7.81 (*d*, *J* = 8, 1 H); 7.89 (*dt*, *J* = 1, 8, 1 H); 7.91 (*d*, *J* = 16, 1 H); 8.20 (*d*, *J* = 8, 1 H); 8.18–8.22 (*m*, 1 H); 8.30 (*d*, *J* = 16, 1 H); 8.37 (*s*, 1 H); 8.76 (*d*, *J* = 5, 1 H).

4. *Synthesis of* **7**–**10**: *General Procedure*. A mixture of **3** or **4**–**6** (10.0 mmol), dry $AcONH_4$ (4.62 g, 60.0 mmol), *N*-[2-(pyrid-2′-yl)-2-oxoethyl]pyridinium iodide (3.26 g, 10.0 mmol), and dry MeOH (100 ml) was refluxed for 24 h. The cooled mixture was filtered and the precipitate washed with cold MeOH.

4′-(4‴-Nitrophenyl)-2,2′:6′,2″-terpyridine (**7**). Yield: 97%. UV (EtOH): 285, 250, 243. IR (film): 1514 (NO_2), 1352 (NO_2). ^{1}H-NMR ((D$_6$)DMSO): 7.53–7.56 (*m*, 2 H); 8.05 (*dt*, *J* = 2, 8, 2 H); 8.22 (*d*, *J* = 9, 2 H); 8.40 (*d*, *J* = 9, 2 H); 8.68 (*d*, *J* = 8,2 H); 8.76 (*s*, 2 H); 8.77 (*d*, *J* = 5, 2 H).

4′-(3‴-Nitrophenyl)-2,2′:6′,2″-terpyridine (**8**). AcOH instead of MeOH was used as solvent and the product crystallized from MeCN. Yield: 56%. UV (EtOH): 310 (sh), 274, 247. IR (film): 1529 (NO_2), 1348 (NO_2). ^{1}H-NMR ((D$_6$)DMSO): 7.56 (*ddd*, *J* = 1, 5, 8, 2 H); 7.89 (*t*, *J* = 8, 1 H); 8.06 (*dt*, *J* = 2, 8, 2 H); 8.40 (*ddd*, *J* = 1, 2, 8, 1 H); 8.41 (*ddd*, *J* = 1, 2, 8, 1 H); 8.66 (*t*, *J* = 2, 1 H); 8.70 (br. *d*, *J* = 8, 2 H); 8.78 (*s*, 2 H); 8.79 (*ddd*, *J* = 1, 2, 5, 2 H).

4′-[4‴-(3⁗-Nitrobenzyloxy)phenyl]-2,2′:6′,2″-terpyridine (**9**). Crystallization from MeCN. Yield: 57%. UV (EtOH): 283, 254, 230 (sh). IR (film): 1529 (NO_2), 1350 (NO_2), 1255 (C–O–C). ^{1}H-NMR ($CDCl_3$): 5.23 (*s*, 2 H); 7.11 (*d*, *J* = 9, 2 H); 7.36 (*dd*, *J* = 5, 8, 2 H); 7.59 (*t*, *J* = 8, 1 H); 7.81 (*d*, *J* = 8, 1 H); 7.88 (*t*, *J* = 8, 2 H); 7.90 (*d*, *J* = 9, 2 H); 8.22 (*d*, *J* = 8, 1 H); 8.37 (*s*, 1 H); 8.67 (*d*, *J* = 8, 2 H); 8.73 (*d*, *J* = 5, 2 H).

4′-[3‴-(3⁗-Nitrobenzyloxy)phenyl]-2,2′:6′,2″-terpyridine (**10**). Yield: 66%. UV (EtOH): 274, 252. IR (film): 1529 (NO_2), 1350 (NO_2), 1263 (C–O–C). ^{1}H-NMR ($CDCl_3$): 5.26 (*s*, 2 H); 7.07 (*d*, *J* = 8,1 H); 7.35–7.39 (*m*, 2 H); 7.45 (*t*, *J* = 8, 1 H); 7.52 (*s*, 1 H); 7.55 (*d*, *J* = 8, 1 H); 7.59 (*t*, *J* = 8, 1 H); 7.83 (*d*, *J* = 8, 1 H); 7.89 (*t*, *J* = 8, 2 H); 8.21 (*d*, *J* = 8, 1 H); 8.38 (*s*, 1 H); 8.67 (*d*, *J* = 8, 2 H); 8.71 (*s*, 2 H); 8.74 (*d*, *J* = 5, 2 H).

5. *Synthesis of* **13**. *4′-(2‴-Phenylethyl)-2,2′:6′,2″-terpyridine* (**12**). $(i\text{-}Pr)_2NH$ (0.85 g, 8.4 mmol) in dry THF (5 ml) was cooled to −70° and deaerated with N_2. BuLi in hexane (3.4 ml, 2.5M) was added and the soln. stirred for 20 min. A soln. of 4′-methyl-2,2′:6′,2″-terpyridine [15] (1.82 g, 7.36 mmol) in dry THF (35 ml) was slowly added and the mixture stirred at −70° for 1 h. Benzyl chloride (0.94 g, 7.4 mmol) was added and stirring continued for 1 h at −70° and then for 1 h at r.t. Then the mixture was washed with sat. NH_4Cl soln. (30 ml), the aq. phase extracted with CH_2Cl_2 (2 × 10 ml) and the combined org. phase dried (Na_2SO_4) and evaporated: 2.35 g (94%). UV (EtOH): 279, 238. IR (film): 1601, 1584, 1558 (arom. C–C). ^{1}H-NMR ($CDCl_3$): 3.05–3.14 (*m*, 4 H); 7.19–7.23 (*m*, 1 H); 7.25–7.31 (*m*, 4 H); 7.34 (*ddd*, *J* = 2, 5, 8, 2 H); 7.86 (*dt*, *J* = 2, 8, 2 H); 8.36 (*s*, 2 H); 8.64 (*d*, *J* = 8, 2 H); 8.71 (*d*, *J* = 5, 2 H).

4′-[2‴-(4⁗-Nitrophenyl)ethyl]-2,2′:6′,2″-terpyridine (**13**). Conc. HNO_3 soln. (60%; 0.52 ml, 6.9 mmol) was added to a soln. of **12** (2.32 g, 6.9 mmol) in THF (25 ml), and the mixture was evaporated. The residue was added in small portions to cold H_2SO_4 (< 10°) and the mixture heated to 50° for 10 min, poured into ice-water, and neutralized with solid Na_2CO_3. After extraction with $CHCl_3$ (3 × 25 ml), the org. fractions were dried (Na_2SO_4) and evaporated. Yield: 84% (sample contained *ca.* 10% of 2⁗-nitrophenyl derivative). UV (EtOH): 278, 239. IR (film): 1518 (NO_2), 1345 (NO_2). ^{1}H-NMR ($CDCl_3$): 3.09–3.20 (*m*, 4 H); 7.32–7.37 (*m*, 4 H); 7.84 (*t*, *J* = 8, 2 H); 8.13 (*d*, *J* = 8, 2 H); 8.31 (*s*, 2 H); 8.63 (*d*, *J* = 8, 2 H); 8.69 (*d*, *J* = 5, 2 H).

6. *Synthesis of* **14**–**19**: *General Procedure*. The 3-chloroperbenzoic acid (2.60 g, 15.1 mmol) was added to a mixture of **7**–**11** or **13** (4.0 mmol) and CH_2Cl_2 (80 ml). After stirring overnight, the mixture was washed with 10% Na_2CO_3 soln. (2 × 50 ml) and H_2O (50 ml), dried (Na_2SO_4), and evaporated.

4′-Phenyl-2,2′:6′,2″-terpyridine N,N″-*Dioxide* (**14**). Crystallization from MeCN/MeOH. Yield: 60%. UV (EtOH): 280, 249. IR (KBr): 1225 (N→O). ^{1}H-NMR ($CDCl_3$): 7.29–7.34 (*m*, 2 H); 7.40 (*t*, *J* = 7, 2 H); 7.43–7.52 (*m*, 3 H); 7.84 (*d*, *J* = 7, 2 H); 8.23 (*dd*, *J* = 2, 8, 2 H); 8.36 (*d*, *J* = 6, 2 H); 9.23 (*s*, 2 H).

4′-(4‴-Nitrophenyl)-2,2′:6′,2″-terpyridine N,N″-*Dioxide* (**15**). Crystallization from MeCN/MeOH. Yield: 92%. UV (EtOH): 282, 241. IR (film): 1514 (NO_2), 1351 (NO_2), 1226 (N→O). ^{1}H-NMR ((D$_6$)DMSO): 7.52–7.58 (*m*, 4 H); 8.11 (*d*, *J* = 7, 2 H); 8.26–8.28 (*m*, 2 H); 8.42 (*d*, *J* = 7, 2 H); 8.43–8.46 (*m*, 2 H); 9.16 (*s*, 2 H).

4′-(3‴-Nitrophenyl)-2,2′:6′,2″-terpyridine N,N″-*Dioxide* (**16**). Crystallization from EtOH/$CHCl_3$. Yield: 89%. UV (EtOH): 280 (sh), 248. IR (film): 1532 (NO_2), 1350 (NO_2), 1240 (N→O). ^{1}H-NMR ((D$_6$)DMSO): 7.52–7.58 (*m*, 4 H); 7.89 (*t*, *J* = 8, 1 H); 8.25–8.28 (*m*, 2 H); 8.31 (*d*, *J* = 8, 1 H); 8.38 (*dd*, *J* = 1, 8, 1 H); 8.43–8.45 (*m*, 2 H); 8.61 (*t*, *J* = 1, 1 H); 9.17 (*s*, 2 H).

4′-[4‴-(3⁗-Nitrobenzyloxy)phenyl]-2,2′:6′,2″-terpyridine N,N″-*Dioxide* (**17**). The residue was triturated with hot MeCN, cooled, and filtered and the solid material washed with MeCN. Yield: 84%. UV (EtOH): 285, 251, 240. IR (film): 1528 (NO_2), 1350 (NO_2), 1274 (C–O–C), 1248 (N→O). ^{1}H-NMR ((D$_6$)DMSO): 5.38 (*s*, 2 H); 7.27 (*d*, *J* = 9, 2 H); 7.51–7.55 (*m*, 4 H); 7.73 (*t*, *J* = 8, 1 H); 7.82 (*d*, *J* = 9, 2 H); 7.96 (*d*, *J* = 8, 1 H); 8.22–8.25 (*m*, 2 H); 8.24 (*d*, *J* = 8, 1 H); 8.37 (*s*, 1 H); 8.42–8.44 (*m*, 2 H); 9.04 (*s*, 2 H).

4′-[3‴-(3⁗-Nitrobenzyloxy)phenyl]-2,2′:6′,2″-terpyridine N,N″-*Dioxide* (**18**). Purification analogous to that of **17**. Yield: 58%. UV (EtOH): 275 (sh), 248. IR (film): 1528 (NO_2), 1350 (NO_2), 1276 (C–O–C), 1248 (N→O).

^{1}H-NMR ($CDCl_3$): 5.24 (*s*, 4 H); 7.07 (*d*, *J* = 8, 1 H); 7.31–7.36 (*m*, 2 H); 7.41 (*t*, *J* = 8, 2 H); 7.43–7.46 (*m*, 2 H); 7.47 (*d*, *J* = 8, 1 H); 7.59 (*t*, *J* = 8, 1 H); 7.83 (*d*, *J* = 8, 1 H); 8.20 (*d*, *J* = 8, 1 H); 8.24 (*dd*, *J* = 2, 8, 2 H); 8.36 (*s*, 1 H); 8.37 (*d*, *J* = 6,2 H); 9.22 (*s*, 2 H).

4′-[2‴-(4⁗-Nitrophenyl)ethyl]-2,2′:6′,2″-terpyridine N,N″-*Dioxide* (**19**). Purification by FC (silica gel, 3% then 5% MeOH in $CHCl_3$). Yield: 53%. UV (EtOH): 275, 244. IR (film): 1516 (NO_2), 1344 (NO_2), 1263 (N→O). ^{1}H-NMR ($CDCl_3$): 3.13–3.20 (*m*, 4 H); 7.29–7.33 (*m*, 2 H); 7.37–7.42 (*m*, 2 H); 7.39 (*d*, *J* = 9, 2 H); 8.15 (*d*, *J* = 9, 2 H); 8.22 (*dd*, *J* = 2, 8, 2 H); 8.34 (*d*, *J* = 6, 2 H); 8.88 (*s*, 2 H).

7. *Synthesis of* **20–25**: *General Procedure.* Me_3SiCN (5.46 g, 55 mmol) was added to **14** or **15–19** (5.5 mmol) in CH_2Cl_2 (60 ml). After 5 min, benzoyl chloride (3.09 g, 22 mmol) was added within 20 min. After stirring overnight, the mixture was evaporated to half volume, 10% K_2CO_3 soln. (160 ml) added, the mixture stirred for 15 min, and the precipitate filtered and washed with H_2O and cold CH_2Cl_2.

4′-Phenyl-2,2′:6′,2″-terpyridine-6,6″-dicarbonitrile (**20**). Yield: 82%. UV (EtOH): 281, 258. IR (film): 2239 (C≡N). ^{1}H-NMR ($CDCl_3$): 7.52 (*t*, *J* = 7, 1 H); 7.59 (*t*, *J* = 7, 2 H); 7.77 (*dd*, *J* = 1, 7, 2 H); 7.91 (*d*, *J* = 7, 2 H); 8.03 (*t*, *J* = 7, 2 H); 8.83 (*s*, 2 H); 8.87 (*dd*, *J* = 1, 7, 2 H).

4′-(4‴-Nitrophenyl)-2,2′:6′,2″-terpyridine-6,6″-dicarbonitrile (**21**). Yield: 98%. UV (EtOH): 286, 257. IR (KBr): 2237 (C≡N), 1520 (NO_2), 1352 (NO_2). ^{1}H-NMR ((D_6)DMSO): 8.22 (*dd*, *J* = 1, 8, 2 H); 8.30 (*d*, *J* = 9, 2 H); 8.33 (*t*, *J* = 8, 2 H); 8.43 (*d*, *J* = 9, 2 H); 8.77 (*s*, 2 H); 8.01 (*dd*, *J* = 1, 8, 2 H).

4′-(3‴-Nitrophenyl)-2,2′:6′,2″-terpyridine-6,6″-dicarbonitrile (**22**). Yield: 71%. UV (EtOH): 285, 253. IR (film): 2233 (C≡N), 1526 (NO_2), 1359 (NO_2). ^{1}H-NMR ((D_6)DMSO): 7.90 (*t*, *J* = 8, 1 H); 8.22 (*dd*, *J* = 1, 8, 2 H); 8.33 (*t*, *J* = 8, 2 H); 8.40–8.43 (*m*, 1 H); 8.45–8.48 (*m*, 1 H); 8.72 (*s*, 1 H); 8.77 (*s*, 2 H); 9.00 (*dd*, *J* = 1, 8, 2 H).

4′-[4‴-(3⁗-Nitrobenzyloxy)phenyl]-2,2′:6′,2″-terpyridine-6,6″-dicarbonitrile (**23**). Yield: 73%. UV (EtOH): 286, 255. IR (KBr): 2236 (C≡N), 1528 (NO_2), 1350 (NO_2), 1264 (C–O–C). ^{1}H-NMR ($CDCl_3$): 5.28 (*s*, 2 H); 7.18 (*d*, *J* = 9, 2 H); 7.62 (*t*, *J* = 8, 1 H); 7.77 (*d*, *J* = 8, 2 H); 7.84 (*d*, *J* = 8, 1 H); 7.91 (*d*, *J* = 9, 2 H); 8.03 (*t*, *J* = 8, 2 H); 8.23 (*d*, *J* = 8, 1 H); 8.39 (*s*, 1 H); 8.80 (*s*, 2 H); 8.87 (*d*, *J* = 8, 2 H).

4′-[3‴-(3⁗-Nitrobenzyloxy)phenyl]-2,2′:6′,2″-terpyridine-6,6″-dicarbonitrile (**24**). Yield: 93%. UV (EtOH): 285 (sh), 259. IR (film): 2233 (C≡N), 1523 (NO_2), 1352 (NO_2), 1273 (C–O–C). ^{1}H-NMR ((D_6)DMSO): 5.44 (*s*, 2 H); 7.20–7.22 (*m*, 1 H); 7.25–7.28 (*m*, 1 H); 7.57 (*d*, *J* = 5, 1 H); 7.62 (*s*, 1 H); 7.76 (*t*, *J* = 8, 1 H); 7.79–7.82 (*m*, 1 H); 8.03 (*dd*, *J* = 1, 7, 1 H); 8.21 (*d*, *J* = 8, 2 H); 8.32 (*t*, *J* = 8, 2 H); 8.40 (*s*, 1 H); 8.67 (*s*, 2 H); 8.99 (*d*, *J* = 8, 2 H).

4′-[2‴-(4⁗-Nitrophenyl)ethyl]-2,2′:6′,2″-terpyridine-6,6″-dicarbonitrile (**25**). After treatment with K_2CO_3, the aq. phase was extracted with $CHCl_3$ (2 × 25 ml), the combined org. phase dried (Na_2SO_4) and evaporated, and the residue crystallized from MeCN/THF. Yield: 69%. UV (EtOH): 288, 258. IR (film): 2237 (C≡N), 1516 (NO_2), 1345 (NO_2). ^{1}H-NMR ($CDCl_3$): 3.18–3.25 (*m*, 4 H); 7.43 (*d*, *J* = 8.7, 2 H); 7.76 (*d*, *J* = 7.8, 2 H); 8.02 (*t*, *J* = 7.8, 2 H); 8.17 (*d*, *J* = 8.7, 2 H); 8.42 (*s*, 2 H); 8.82 (*d*, *J* = 7.8, 2 H).

8. *Synthesis of* **26–31**: *General Procedure.* A suspension of **20** or **21–25** (1.9 mmol) and dry THF (15 ml) was deaerated with N_2. BH_3·THF (1M; 25 ml, 25.0 mmol) was added within 10 min. After stirring overnight, excess BH_3 was distroyed by addition of MeOH, the mixture evaporated, and the residue dissolved in MeOH saturated with dry HCl (30 ml). After stirring for 1 h, the cooled mixture was filtered and the solid material washed with cold MeOH. A mixture of this material (0.90 mmol), $BrCH_2COOEt$ (1.44 g, 7.4 mmol; $BrCH_2COOMe$ or $BrCH_2COO(t\text{-Bu})$ for **26** and **31**, resp.), dry $(i\text{-Pr})_2EtN$ (3.59 g, 28 mmol), and dry MeCN (15 ml) was refluxed for 20 h. After evaporation, the residue was dissolved in $CHCl_3$ (40 ml) the soln. washed with H_2O (3 × 20 ml) and dried (Na_2SO), and the product purified by FC (silica gel).

Tetramethyl 2,2′,2″,2‴-[(4′-Phenyl-2,2′:6′,2″-terpyridine-6,6″-diyl)bis(methylenenitrilo)]tetrakis(acetate) (**26**). FC: 2% MeOH in $CHCl_3$. Yield: 36%. UV (EtOH): 281, 253. IR (film): 1747 (C=O), 1201 (C–O). ^{1}H-NMR ($CDCl_3$): 3.70 (*s*, 12 H); 3.73 (*s*, 8 H); 4.19 (*s*, 4 H); 7.47 (*t*, *J* = 7, 1 H); 7.55 (*t*, *J* = 7, 2 H); 7.60 (*d*, *J* = 8, 2 H); 7.87 (*t*, *J* = 8, 2 H); 7.90 (*d*, *J* = 7, 2 H); 8.55 (*d*, *J* = 8, 2 H); 8.72 (*s*, 2 H). UV (EtOH): 281, 253. IR (film): 1747 (C=O), 1201 (C–O).

Tetraethyl 2,2′,2″,2‴-{[4′-(4‴-Nitrophenyl)-2,2′:6′,2″-terpyridine-6,6″-diyl]bis(methylenenitrilo)}tetrakis(acetate) (**27**). FC: petroleum ether (b.p. 40–60°)/AcOEt/Et_3N 3:5:1 eluted first **27** (yield: 14%), later the corresponding amino derivative **32** (yield: 12%). UV (EtOH): 287, 233. IR (film): 1739 (C=O), 1523 (NO_2), 1348 (NO_2), 1193 (C–O). ^{1}H-NMR ($CDCl_3$): 1.25 (*t*, *J* = 7, 12 H); 3.71 (*s*, 8 H); 4.18 (*q*, *J* = 7, 8 H); 4.21 (*s*, 4 H); 7.66 (*dd*, *J* = 2, 8, 2 H); 7.90 (*t*, *J* = 8, 2 H); 8.06 (*d*, *J* = 9, 2 H); 8.42 (*d*, *J* = 9, 2 H); 8.58 (*dd*, *J* = 2, 8, 2 H); 8.76 (*s*, 2 H).

Tetraethyl 2,2′,2″,2‴-{[4′-(3‴-Nitrophenyl)-2,2′:6′,2″-terpyridine-6,6″-diyl]bis(methylenenitrilo)}tetrakis(acetate) (**28**). FC: 2% MeOH in $CHCl_3$. Yield: 16%. UV (EtOH: 282, 247. IR (film): 1744 (C=O), 1532 (NO_2), 1349 (NO_2), 1193 (C–O). ^{1}H-NMR ($CDCl_3$): 1.24 (*t*, *J* = 7.3, 12 H); 3.70 (*s*, 8 H); 4.17 (*q*, *J* = 7.3, 8 H); 4.21 (*s*,

4 H); 7.68 (*d*, *J* = 7.8, 2 H); 7.74 (*t*, *J* = 7.8, 1 H); 7.89 (*t*, *J* = 7.8, 2 H); 8.23 (*d*, *J* = 7.8, 1 H); 8.34 (*d*, *J* = 1 H); 8.56 (*d*, *J* = 7.8, 2 H); 8.74 (*s*, 1 H); 8.74 (*s*, 2 H).

Tetraethyl 2,2′,2″,2‴-{{4′-[4‴-(3⁗-Nitrobenzyloxy)phenyl]-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetate) (**29**). FC: 2% then 5% MeOH in $CHCl_3$. Yield: 57%. UV (EtOH): 288, 257. IR (film): 1738 (C=O), 1732 (C=O), 1532 (NO_2), 1350 (NO_2), 1246 (C–O–C), 1193 (C–O). ^{1}H-NMR ($CDCl_3$): 1.23 (*t*, *J* = 7, 12 H); 3.70 (*s*, 8 H); 4.17 (*q*, *J* = 7, 8 H); 4.22 (*s*, 4 H); 5.24 (*s*, 2 H); 7.12 (*d*, *J* = 9, 2 H); 7.60 (*t*, *J* = 8, 1 H); 7.71 (*d*, *J* = 8, 2 H); 7.83 (*d*, *J* = 8, 1 H); 7.87 (*t*, *J* = 8, 2 H); 7.88 (*d*, *J* = 9, 2 H); 8.22 (*d*, *J* = 8, 1 H); 8.35 (*s*, 1 H); 8.54 (*d*, *J* = 8, 2 H); 8.69 (*s*, 2 H).

Tetraethyl 2,2′,2″,2‴-{{4′-[3‴-(3⁗-Nitrobenzyloxy)phenyl]-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetate) (**30**). FC: 2% MeOH in $CHCl_3$. Yield: 38%. UV (EtOH): 280, 254. IR (film): 1744 (C=O), 1532 (NO_2), 1350 (NO_2), 1265 (C–O–C), 1193 (C–O). ^{1}H-NMR ($CDCl_3$): 1.22 (*t*, *J* = 7, 12 H); 3.71 (*s*, 8 H); 4.15 (*q*, *J* = 7, 8 H); 4.20 (*s*, 4 H); 5.32 (*s*, 2 H); 7.09 (*d*, *J* = 8, 1 H); 7.47 (*t*, *J* = 8, 1 H); 7.51–7.54 (*m*, 2 H); 7.60 (*t*, *J* = 8, 1 H); 7.64 (*d*, *J* = 8, 2 H); 7.86 (*d*, *J* = 8, 1 H); 7.87 (*t*, *J* = 8, 2 H); 8.22 (*d*, *J* = 8, 1 H); 8.40 (*s*, 1 H); 8.55 (*d*, *J* = 8, 2 H); 8.69 (*s*, 2 H).

*Tetra(*tert*-butyl) 2,2′,2″,2‴-{{4′-[2‴-(4⁗-Nitrophenyl)ethyl]-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetate)* (**31**). FC: petroleum ether (b.p. 40–60°)/AcOEt/Et_3N 5:1:1. Yield: 40%. UV (EtOH): 282, 242. IR (film): 1738 (C=O), 1522 (NO_2), 1346 (NO_2), 1144 (C–O). ^{1}H-NMR ($CDCl_3$): 1.48 (*s*, 36 H); 3.10–3.22 (*m*, 4 H); 3.55 (*s*, 8 H); 4.17 (*s*, 4 H); 7.44 (*d*, *J* = 8.7, 2 H); 7.69 (*d*, *J* = 7.6, 2 H); 7.85 (*t*, *J* = 7.6, 2 H); 8.18 (*d*, *J* = 8.7, 2 H); 8.35 (*s*, 2 H); 8.50 (*d*, *J* = 7.6, 2 H).

9. *Synthesis of* **32–36**: *General Methods. Method A.* $SnCl_2 \cdot 2H_2O$ (0.22 g, 1.0 mmol) was added to a hot (70–80°) soln. of nitro compound (0.16 mmol) in abs. EtOH (10 ml). After stirring at 70–80° for 0.5 h, the cooled mixture was poured to an ice-cold mixture of DTPA (1.55 g, 3.94 mmol) and H_2O (25 ml). The mixture was neutralized with sat. $NaHCO_3$ soln., the aq. phase extracted with $CHCl_3$ (3 × 20 ml), and the combined org. phase dried (Na_2SO_4).

Method B. The nitro compound (0.07 mmol) was dissolved in dry MeOH (2 ml), and 10% Pd/C (10 mg) was added followed by slow addition of $NaBH_4$ (4 mg, 0.1 mmol). After 1 h stirring, the mixture was filtered, the filtrate evaporated, the residue dissolved in H_2O, and the product extracted with $CHCl_3$. The $CHCl_3$ phase was dried (Na_2SO_4).

Method C. A mixture of nitro compound (0.48 mmol), 10% Pd/C (50 mg), and MeOH (20 ml) was stirred under H_2 (6.8 atm) for 2 h. After filtration, the filtrate was evaporated.

Tetraethyl 2,2′,2″,2‴-{[4′-(4‴-Aminophenyl)-2,2′:6′,2″-terpyridine-6,6″-diyl]bis(methylenenitrilo)}tetrakis(acetate) (**32**). *Method A.* Yield: 100% (16% with *Method B*). UV (EtOH): 330 (sh), 291. IR ($CHCl_3$): 1742 (C=O), 1194 (C–O). ^{1}H-NMR ($CDCl_3$): 1.25 (*t*, *J* = 7, 12 H); 3.73 (*s*, 8 H); 4.19 (*q*, *J* = 7, 8 H); 4.25 (*s*, 4 H); 6.81 (*d*, *J* = 8, 2 H); 7.65 (*dd*, *J* = 1, 8, 2 H); 7.89 (*t*, *J* = 8, 2 H); 8.09 (*d*, *J* = 8, 2 H); 8.59 (*dd*, *J* = 1, 8, 2 H); 8.81 (*s*, 2 H).

Tetraethyl 2,2′,2″,2‴-{[4′-(3‴-Aminophenyl)-2,2′:6′,2″-terpyridine-6,6″-diyl]bis(methylenenitrilo)}tetrakis(acetate) (**33**). *Method A.* Purification by FC (silica gel, petroleum ether (b.p. 40–60°)/AcOEt/Et_3N 5:3:2). Yield: 100% (79% with *Method B*). UV (EtOH): 315 (sh), 280, 248. IR (film): 1743 (C=O), 1196 (C–O). ^{1}H-NMR ((D_6)DMSO): 1.13 (*t*, *J* = 7.3, 12 H); 3.64 (*s*, 8 H); 4.05 (*q*, *J* = 7.3, 8 H); 4.11 (*s*, 4 H); 6.73 (*d*, *J* = 7.9, 1 H); 6.98 (*d*, *J* = 7.9, 1 H); 7.07 (*s*, 1 H); 7.23 (*t*, *J* = 7.9, 1 H); 7.63 (*d*, *J* = 7.8, 2 H); 8.02 (*t*, *J* = 7.8, 2 H); 8.54 (*d*, *J* = 7.8, 2 H); 8.57 (*s*, 2 H).

Tetraethyl 2,2′,2″,2‴-{{4′-[4‴-(3⁗-Aminobenzyloxy)phenyl]-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetate) (**34**). *Method A.* Purification by FC (silica gel, 2% then 10% MeOH in $CHCl_3$). Yield: 69%. UV (EtOH): 335 (sh), 294, 233. IR (film): 1738 (C=O), 1730 (C=O), 1194 (C–O). ^{1}H-NMR ($CDCl_3$): 1.20 (*t*, *J* = 7, 12 H); 3.84 (*s*, 8 H); 4.14 (*q*, *J* = 7, 8 H); 4.67 (*s*, 4 H); 5.05 (*s*, 2 H); 6.67 (*d*, *J* = 8, 1 H); 6.81 (*s*, 1 H); 6.83 (*d*, *J* = 8, 1 H); 7.10 (*d*, *J* = 8, 2 H); 7.16 (*t*, *J* = 8, 1 H); 7.63 (*d*, *J* = 8, 2 H); 7.74 (*d*, *J* = 8, 2 H); 7.97 (*t*, *J* = 8, 2 H); 8.16 (*d*, *J* = 8, 2 H); 8.17 (*s*, 2 H).

Tetraethyl 2,2′,2″,2‴-{{4′-[3‴-(3⁗-Aminobenzyloxy)phenyl]-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetate) (**35**). *Method A.* Purification by FC (silica gel, 2% then 10% MeOH in $CHCl_3$). Yield: 64%. UV (EtOH): 288, 241. IR (film): 1738 (C=O), 1732 (C=O), 1195 (C–O). ^{1}H-NMR ((D_6)DMSO): 1.12 (*t*, *J* = 7, 12 H); 3.65 (*s*, 8 H); 4.04 (*q*, *J* = 7, 8 H); 4.10 (*s*, 4 H); 5.09 (*s*, 2 H); 6.53 (*d*, *J* = 8, 1 H); 6.64 (*d*, *J* = 8, 1 H); 6.69 (*s*, 1 H); 7.05 (*t*, *J* = 8, 1 H); 7.17 (*d*, *J* = 8, 1 H); 7.43 (*d*, *J* = 8, 1 H); 7.44 (*s*, 1 H); 7.52 (*t*, *J* = 8, 1 H); 7.63 (*d*, *J* = 8, 2 H); 8.02 (*t*, *J* = 8, 2 H); 8.54 (*d*, *J* = 8, 2 H); 8.62 (*s*, 2 H).

*Tetra(*tert*-butyl) 2,2′,2″,2‴-{{4′-[2‴-(4⁗-Aminophenyl)ethyl]-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetate)* (**36**). *Method C.* Purification by FC (silica gel, petroleum ether (b.p. 40–60°)/AcOEt/Et_3N 5:1:1). Yield: 48%. UV (EtOH): 286, 241. IR (film): 1738 (C=O), 1733 (C=O), 1143 (C–O). ^{1}H-NMR

($CDCl_3$): 1.47 (*s*, 36 H); 2.92–3.08 (*m*, 4 H); 3.55 (*s*, 8 H); 4.17 (*s*, 4 H); 6.66 (*d*, *J* = 8.6, 2 H); 7.07 (*d*, *J* = 8.6, 2 H); 7.68 (*d*, *J* = 7.8, 2 H); 7.83 (*t*, *J* = 7.8, 2 H); 8.33 (*s*, 2 H); 8.49 (*d*, *J* = 7.8, 2 H).

10. *Synthesis of* **37**–**43**: *General Method.* 10.1. *Ethyl and Methyl Esters.* A mixture of tetraethyl or tetramethyl ester **26** or **32**–**35**, 0.15 mmol, 0.5M KOH/EtOH (6 ml), and H_2O (1 ml) was stirred for 2 h at r.t. The mixture was neutralized with 1M HCl, evaporated, and the residue used in the next step without further purification.

10.2. tert-*Butyl Esters.* A soln. of tetra(*tert*-butyl) ester **31** or **36** (0.35 mmol) in CF_3COOH (5 ml) was stirred for 2 h at r.t. After evaporation, the mixture was triturated with Et_2O and filtered.

2,2′,2″,2‴-[(4′-Phenyl-2,2′:6′,2″-terpyridine-6,6″-diyl)bis(methylenenitrilo)]tetrakis(acetic Acid) (**37**). UV (H_2O): 280, 250. IR (KBr): 1728 (C=O), 1612 (C=O), 1399 (C–O), 1219 (C–O). ^{1}H-NMR ((D_6)DMSO): 3.61 (*s*, 8 H); 4.14 (*s*, 8 H); 7.56 (*t*, *J* = 7, 1 H); 7.62 (*t*, *J* = 7, 2 H); 7.65 (*d*, *J* = 8, 2 H); 7.94 (*d*, *J* = 7, 2 H); 8.04 (*t*, *J* = 8, 2 H); 8.56 (*d*, *J* = 8, 2 H); 8.70 (*s*, 2 H).

2,2′,2″,2‴-{[4′-(4‴-Aminophenyl)-2,2′:6′,2″-terpyridine-6,6″-diyl]bis(methylenenitrilo)}tetrakis(acetic Acid) (**38**). UV (H_2O): 325, 292, 233. IR (KBr): 1728 (C=O), 1684 (C=O), 1625 (C=O), 1396 (C–O), 1199 (C–O). ^{1}H-NMR ((D_6)DMSO): 3.82 (*s*, 8 H); 4.31 (*s*, 4 H); 6.90 (*d*, *J* = 8, 2 H); 7.67 (*d*, *J* = 8, 2 H); 7.80 (*d*, *J* = 8, 2 H); 8.08 (*t*, *J* = 8, 2 H); 8.60 (*d*, *J* = 8, 2 H); 8.67 (*s*, 2 H).

2,2′,2″,2‴-{[4′-(3‴-Aminophenyl)-2,2′:6′,2″-terpyridine-6,6″-diyl]bis(methylenenitrilo)}tetrakis(acetic Acid) (**39**). UV (H_2O): 325, 282, 245. IR (KBr): 1719 (C=O), 1623 (C=O), 1395 (C–O), 1214 (C–O). ^{1}H-NMR (D_2O): 3.31 (*s*, 8 H); 4.00 (*s*, 4 H); 6.91 (*d*, *J* = 7, 1 H); 7.28–7.52 (*m*, 3 H); 7.70 (*d*, *J* = 7, 2 H); 7.97 (*t*, *J* = 7, 2 H); 8.07 (*d*, *J* = 7, 2 H); 8.23 (*s*, 2 H).

2,2′,2″,2‴-{{4′-[4‴-(3‴′-Aminobenzyloxy)phenyl]-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetic Acid) (**40**). UV (H_2O): 325 (sh), 291, 232. IR (KBr): 1627 (C=O), 1604 (C=O), 1398 (C–O), 1260 (C–O–C), 1187 (C–O). ^{1}H-NMR ((D_6)DMSO): 3.76 (*s*, 8 H); 4.27 (*s*, 4 H); 5.19 (*s*, 2 H); 6.98 (*d*, *J* = 7, 1 H); 7.09 (*d*, *J* = 7, 1 H); 7.10 (*s*, 1 H); 7.25 (*d*, *J* = 7, 2 H); 7.31 (*t*, *J* = 7, 1 H); 7.67 (*d*, *J* = 7, 2 H); 7.96 (*d*, *J* = 8, 2 H); 8.07 (*t*, *J* = 8, 2 H); 8.60 (*d*, *J* = 8, 2 H); 8.70 (*s*, 2 H).

2,2′,2″,2‴-{{4′-[3‴-(3‴′-Aminobenzyloxy)phenyl]-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetic Acid) (**41**). UV (H_2O): 315 (sh), 283, 240 (sh). IR (KBr): 1630 (C=O), 1607 (C=O), 1395 (C–O), 1268 (C–O–C), 1205 (C–O). ^{1}H-NMR ((D_6)DMSO): 3.79 (*s*, 8 H); 4.30 (*s*, 4 H); 5.26 (*s*, 2 H); 7.07 (*d*, *J* = 8, 1 H); 7.19–7.24 (*m*, 3 H); 7.37 (*t*, *J* = 8, 1 H); 7.55–7.59 (*m*, 3 H); 7.68 (*d*, *J* = 8, 2 H); 8.09 (*t*, *J* = 8, 2 H); 8.61 (*d*, *J* = 8, 2 H); 8.72 (*s*, 2 H).

2,2′,2″,2‴-{{4′-[2‴-(4‴′-Nitrophenyl)ethyl]-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis-(acetic Acid) (**42**). UV (H_2O): 284, 243. IR (KBr): 1732 (C=O), 1519 (NO_2), 1347 (NO_2), 1195 (C–O). ^{1}H-NMR ((D_6)DMSO): 3.15–3.24 (*m*, 4 H); 3.89 (*s*, 8 H); 4.37 (*s*, 4 H); 7.64 (*d*, *J* = 8.5, 2 H); 7.67 (*d*, *J* = 7.8, 2 H); 8.08 (*t*, *J* = 7.8, 2 H); 8.17 (*d*, *J* = 8.5, 2 H); 8.38 (*s*, 2 H); 8.57 (*d*, *J* = 7.8, 2 H).

2,2′,2″,2‴-{{4′-[2‴-(4‴′-Aminophenyl)ethyl]-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis-(acetic Acid) (**43**). UV (H_2O): 287, 242. IR (KBr): 1734 (C=O), 1199 (C–O). ^{1}H-NMR ((D_6)DMSO): 3.08–3.15 (*m*, 4 H); 3.83 (*s*, 8 H); 4.32 (*s*, 4 H); 7.22 (*d*, *J* = 8.4, 2 H); 7.43 (*d*, *J* = 8.4, 2 H); 7.66 (*d*, *J* = 7.8, 2 H); 8.07 (*t*, *J* = 7.8, 2 H); 8.37 (*s*, 2 H); 8.56 (*d*, *J* = 7.8, 2 H).

11. *Synthesis of* **50**. N,N′-*[2,2′-(Pyridine-2,6-diyl)bis(2-oxoethyl)]bis(pyridinium Iodide)* (**44**). A mixture of 2,6-diacetylpyridine (1.63 g, 10.0 mmol), I_2 (5.08 g, 20.0 mmol), and pyridine (25 ml) was refluxed for 2 h. The cold mixture was filtered and the product washed with pyridine: 5.24 g (91%). UV (EtOH): 427, 265 (sh), 245 (sh), 218. IR (KBr): 1716 (C=O). ^{1}H-NMR ((D_6)DMSO): 6.70 (*s*, 4 H); 8.35 (*dd*, *J* = 7, 8, 4 H); 8.44–8.45 (*m*, 3 H); 8.80 (*t*, *J* = 8, 2 H); 9.13 (*d*, *J* = 7, 4 H).

Diammonium 4,4″-Diphenyl-2,2′:6′,2″-terpyridine-6,6″-dicarboxylate (**45**). A mixture of **44** (2.87 g, 5.00 mmol), sodium 2-oxo-4-phenylbut-3-enoate [22] (1.98 g, 10.0 mmol), $AcONH_4$ (8.86 g, 115 mmol), and AcOH (50 ml) was refluxed for 5 h. The brown soln. was cooled and the precipitated product filtered and washed with AcOH: 1.64 g (65%). UV (H_2O): 242. IR (KBr): 1577, 1406 (COO). ^{1}H-NMR ((D_6)DMSO): 7.60 (*t*, *J* = 7, 2 H); 7.63 (*t*, *J* = 7, 4 H); 8.04 (*d*, *J* = 7, 4 H); 8.27 (*t*, *J* = 8, 1 H); 8.40 (*d*, *J* = 2, 2 H); 8.70 (*d*, *J* = 8, 2 H); 9.17 (*d*, *J* = 2, 2 H).

Dimethyl 4,4″-Diphenyl-2,2′:6′,2″-terpyridine-6,6″-dicarboxylate (**46**). Compound **45** (1.64 g, 3.23 mmol) was dissolved in 2M NaOH (400 ml) and the pH adjusted to 1 with 6M HCl. The diacid was filtered and washed with H_2O. A mixture of dry diacid (0.77 g, 1.6 mmol), MeOH (10 ml), and conc. H_2SO_4 soln. (0.05 ml) was refluxed for 24 h. The cold mixture was filtered and the product crystallized from CH_2Cl_2: 0.49 g (61%). M.p. 285.5–286.5°. UV (EtOH): 305 (sh), 252. IR (KBr): 1741 (C=O), 1725 (C=O), 1255 (C–O), 1144 (C–O). ^{1}H-NMR ($CDCl_3$): 4.09 (*s*, 6 H); 7.52–7.58 (*m*, 6 H); 7.86 (*dd*, *J* = 1, 7, 4 H); 8.06 (*t*, *J* = 8, 1 H); 8.44 (*d*, *J* = 2, 2 H); 8.67 (*d*, *J* = 8, 2 H); 9.07 (*d*, *J* = 2, 2 H).

4,4″-Diphenyl-2,2′:6′,2″-terpyridine-6,6′-bis(methanol) (**47**). $NaBH_4$ (0.13 g, 3.4 mmol) was added to a suspension of **46** (0.40 g, 0.80 mmol) in abs. EtOH (10 ml). After stirring for 2 h at r.t., the mixture was refluxed for 15 h. The soln. was evaporated, sat. $NaHCO_3$ soln. (5 ml) added, and the soln. brought to boiling. H_2O (15 ml) was added and the mixture allowed to stand overnight in the cold. The product was filtered and crystallized from MeOH: 0.17 g (48%). M.p. 218–219°. UV (EtOH): 302, 246. IR (film): 1610, 1579, 1551 (arom. C–C). H-NMR ((D_6)DMSO): 4.77 (*d*, *J* = 5, 4 H); 5.61 (*t*, *J* = 5, 2 H); 7.55 (*t*, *J* = 7, 2 H); 7.61 (*t*, *J* = 7, 4 H); 7.87 (*d*, *J* = 2, 2 H); 7.94 (*d*, *J* = 7, 4 H); 8.14 (*t*, *J* = 8, 1 H); 8.49 (*d*, *J* = 8, 2 H); 8.81 (*d*, *J* = 2, 2 H).

6,6′-Bis(bromomethyl)-4,4″-diphenyl-2,2′:6′,2″-terpyridine (**48**). A soln. of PBr_3 (0.12 g, 0.44 mmol) in $CHCl_3$ (1 ml) was added to a soln. of **47** (0.13 g, 0.29 mmol) in $CHCl_3$ (17 ml). The mixture was refluxed for 11 h, whereafter the mixture was neutralized with 5% $NaHCO_3$ soln. The aq. layer was extracted with $CHCl_3$ (3 × 20 ml), the combined org. phase dried (Na_2SO_4) and evaporated, and the residue crystallized from CH_2Cl_2: 48 mg (29%). M.p. 253.5–255.5°. UV (EtOH): 303, 249. IR (film): 1610, 1575, 1549 (arom. C–C). ^{1}H-NMR ($CDCl_3$): 4.73 (*s*, 4 H); 7.49 (*t*, *J* = 7, 2 H); 7.54 (*t*, *J* = 7, 4 H); 7.73 (*d*, *J* = 2, 2 H); 7.80 (*d*, *J* = 7, 4 H); 8.01 (*t*, *J* = 8, 1 H); 8.57 (*d*, *J* = 8, 2 H); 8.79 (*d*, *J* = 2, 2 H).

*Tetra(*tert*-butyl) 2,2′,2″,2‴-[(4,4″-Diphenyl-2,2′:6′,2″-terpyridine-6,6″-diyl)bis(methylenenitrilo)]tetrakis(acetate)* (**49**). A mixture of **48** (50 mg, 88 μmol), di(*tert*-butyl) iminobis(acetate) (44 mg, 180 μmol), dry K_2CO_3 (120 mg, 0.87 mmol), and dry MeCN (5 ml) was refluxed overnight. The mixture was evaporated and the suspension of the residue in $CHCl_3$ (10 ml) washed with 5% $NaHCO_3$ soln. (2 × 5 ml) and H_2O (2 × 10 ml) and dried (Na_2SO_4): 47 mg (59%). UV (EtOH): 304, 247. ^{1}H-NMR ($CDCl_3$): 1.47 (*s*, 36 H); 3.61 (*s*, 8 H); 4.24 (*s*, 4 H); 7.46 (*t*, *J* = 7, 2 H); 7.51 (*t*, *J* = 7, 4 H); 7.84 (*d*, *J* = 7, 4 H); 7.92 (*d*, *J* = 2, 2 H); 7.95 (*t*, *J* = 8, 1 H); 8.52 (*d*, *J* = 8, 2 H); 8.79 (*d*, *J* = 2, 2 H).

2,2′,2″,2‴-[(4,4″-Diphenyl-2,2′:6′,2″-terpyridine-6,6″-diyl)bis(methylenenitrilo)]tetrakis(acetic Acid) (**50**). For 1.5 h, **49** (47 mg, 52 μmol) was stirred in CF_3COOH (3 ml). CF_3COOH was evaporated, the residue triturated with Et_2O, and the product filtered: 35 mg (100%). UV (H_2O): 310, 247. IR (KBr): 1734 (C=O), 1395 (C–O), 1200 (C–O). ^{1}H-NMR ((D_6)DMSO): 3.71 (*s*, 8 H); 4.27 (*s*, 4 H); 7.56 (*t*, *J* = 7, 2 H); 7.61 (*t*, *J* = 7, 4 H); 7.96 (*d*, *J* = 7, 4 H); 8.04 (*d*, *J* = 1, 2 H); 8.18 (*t*, *J* = 8, 1 H); 8.51 (*d*, *J* = 8, 2 H); 8.85 (*d*, *J* = 1, 2 H).

12. *Europium(III) and Terbium(III) Chelates: General Procedure.* Tetraacid **37–43** or **50** (0.15 mmol) was dissolved in H_2O (2 ml) and the pH adjusted to 6.5 with solid $NaHCO_3$. $EuCl_3$ or $TbCl_3$ (0.16 mmol) in H_2O (1 ml) was added within 15 ml and the pH maintained at 5–7. After stirring for 1.5 h, the pH was raised to 8.5 with 1M NaOH, the precipitate filtered off, the filtrate triturated with acetone, and the precipitate filtered and washed with acetone. The product was used in the next step without further purification.

{2,2′,2″,2‴-[(4′-Phenyl-2,2′:6′,2″-terpyridine-6,6″-diyl)bis(methylenenitrilo)]tetrakis(acetato)}europium(III) (**51**). UV (H_2O): 342, 331, 295, 287.

{2,2′,2″,2‴-[(4′-Phenyl-2,2′:6′,2″-terpyridine-6,6″-diyl)bis(methylenenitrilo)]tetrakis(acetato)}terbium(III) (**52**). UV (H_2O): 342, 331, 295, 287 (sh).

{2,2′,2″,2‴-[(4,4″-Diphenyl-2,2′:6′,2″-terpyridine-6,6″-diyl)bis(methylenenitrilo)]tetrakis(acetato)}europium(III) (**53**). UV (H_2O): 342, 280 (sh), 245.

{2,2′,2″,2‴-[(4,4″-Diphenyl-2,2′:6′,2″-terpyridine-6,6″-diyl)bis(methylenenitrilo)]tetrakis(acetato)}terbium(III) (**54**). UV (H_2O): 342, 278, 247.

{2,2′,2″,2‴-{[4′-(4‴-Aminophenyl)-2,2′:6′,2″-terpyridine-6,6″-diyl]bis(methylenenitrilo)}tetrakis(acetato)}europium(III) (**55**). UV (H_2O): 332, 295, 235.

{2,2′,2″,2‴-{[4′-(4‴-Aminophenyl)-2,2′:6′,2″-terpyridine-6,6″-diyl]bis(methylenenitrilo)}tetrakis(acetato)}terbium(III) (**56**). UV (H_2O): 335, 295, 237.

{2,2′,2″,2‴-{[4′-(3‴-Aminophenyl)-2,2′:6′,2″-terpyridine-6,6″-diyl]bis(methylenenitrilo)}tetrakis(acetato)}europium(III) (**57**). UV (H_2O): 333, 295, 236.

{2,2′,2″,2‴-{[4′-(3‴-Aminophenyl)-2,2′:6′,2″-terpyridine-6,6″-diyl]bis(methylenenitrilo)}tetrakis(acetato)}terbium(III) (**58**). UV (H_2O): 335, 298, 240.

{2,2′,2″,2‴-{{4′-[4‴-(3⁗-Aminobenzyloxy)phenyl]-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetato)}europium(III) (**59**). UV (H_2O): 330, 297, 235.

{2,2′,2″,2‴-{{4′-[4‴-(3⁗-Aminobenzyloxy)phenyl]-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetato)}terbium(III) (**60**). UV (H_2O): 330, 296, 235.

{2,2′,2″,2‴-{{4′-[3‴-(3⁗-Aminobenzyloxy)phenyl]-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetato)}europium(III) (**61**). UV (H_2O): 328, 294, 240 (sh).

{2,2′,2″,2‴-{{4′-[3‴-(3⁗-Aminobenzyloxy)phenyl]-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetato)}terbium(III) (**62**). UV (H_2O): 330, 295, 240 (sh).

{2,2′,2″,2‴-{{4′-[2‴-(4⁗-Nitrophenyl)ethyl]-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetato)}europium(III) (**63**). UV (H_2O): 335, 324, 294, 283, 236.

{2,2′,2″,2‴-{{4′-[2‴-(4⁗-Nitrophenyl)ethyl]-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetato)}terbium(III) (**64**). UV (H_2O): 335, 324, 294, 286, 237.

{2,2′,2″,2‴-{{4′-[2‴-(4⁗-Aminophenyl)ethyl]-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetato)}europium(III) (**65**). UV (H_2O): 335, 325, 294, 284, 237.

{2,2′,2″,2‴-{{4′-[2‴-(4⁗-Aminophenyl)ethyl]-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetato)}terbium(III) (**66**). UV (H_2O): 335, 325, 293, 284, 237.

13. *Activation of Amino Groups with Thiophosgene: General Procedure.* An aq. soln. (3.5 ml) of amino chelate **55**–**62**, **65** or **66** (0.15 mmol) was added within 15 min to a mixture of thiophosgene (41 µl, 0.53 mmol), $NaHCO_3$, (55 mg, 0.66 mmol), and $CHCl_3$ (3.5 ml). After stirring for 1 h, the H_2O phase was washed with $CHCl_3$ (3 × 2 ml), acetone was added to the aq. soln. and the precipitate filtered and washed with acetone.

{2,2′,2″,2‴-{[4′-(4‴-Isothiocyanatophenyl)-2,2′:6′,2″-terpyridine-6,6″-diyl]bis(methylenenitrilo)}tetrakis(acetato)}europium(III) (**67**). UV (H_2O): 330, 297, 234. IR (KBr): 2062 (S=C=N).

{2,2′,2″,2‴-{[4′-(4‴-Isothiocyanatophenyl)-2,2′:6′,2″-terpyridine-6,6″-diyl]bis(methylenenitrilo)}tetrakis(acetato)}terbium(III) (**68**). UV (H_2O): 320 (sh), 297, 230. IR (KBr): 2082 (S=C=N).

{2,2′,2″,2‴-{[4′-(3‴-Isothiocyanatophenyl)-2,2′:6′,2″-terpyridine-6,6″-diyl]bis(methylenenitrilo)}tetrakis(acetato)}europium(III) (**69**). UV (H_2O): 332, 295, 286, 240. IR (KBr): 2115 (S=C=N).

{2,2′,2″,2‴-{[4′-(3‴-Isothiocyanatophenyl)-2,2′:6′,2″-terpyridine-6,6″-diyl]bis(methylenenitrilo)}tetrakis(acetato)}terbium(III) (**70**). UV (H_2O): 330, 294, 285, 240 (sh). IR (KBr): 2130 (S=C=N).

{2,2′,2″,2‴-{{4′-[4‴-(3⁗-Isothiocyanatobenzyloxy)phenyl]-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetato)}europium(III) (**71**). UV (H_2O): 330, 296, 237 (sh). IR (KBr): 2107 (S=C=N).

{2,2′,2″,2‴-{{4′-[4‴-(3⁗-Isothiocyanatobenzyloxy)phenyl]-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetato)}terbium(III) (**72**). UV (H_2O): 330 (sh), 296, 237. IR (KBr): 2113 (S=C=N).

{2,2′,2″,2‴-{{4′-[3‴-(3⁗-Isothiocyanatobenzyloxy)phenyl]-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetato)}europium(III) (**73**). UV (H_2O): 328, 295, 286, 267 (sh). IR (KBr): 2118 (S=C=N).

{2,2′,2″,2‴-{{4′-[3‴-(3⁗-Isothiocyanatobenzyloxy)phenyl]-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetato)}terbium(III) (**74**). UV (H_2O): 328, 295, 285. IR (KBr): 2121 (S=C=N).

{2,2′,2″,2‴-{{4′-[2‴-(4⁗-Isothiocyanatophenyl)ethyl]-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetato)}europium(III) (**75**). UV (H_2O): 337, 328, 294, 283, 271, 240 (sh). IR (KBr): 2038 (S=C=N).

{2,2′,2″,2‴-{{4′-[2‴-(4⁗-Isothiocyanatophenyl)ethyl]-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}-tetrakis(acetato)}terbium(III) (**76**). UV (H_2O): 337, 325, 294, 284, 273, 238. IR (KBr): 2099 (S=C=N).

14. *Activation of Amino Groups with 2,4,6-Trichloro-1,3,5-triazine: General Procedure.* A mixture of 2,4,6-trichloro-1,3,5-triazine (18 mg, 0.1 mmol), acetone (1.0 ml), and H_2O (1.0 ml) was added to a soln. of amino chelate **55**–**62**, **65**, or **66** (0.1 mmol) and 0.1M NaOAc (1.5 ml, pH 4.9). After stirring for 30 min, acetone was added to the mixture and the precipitate filtered and washed with acetone.

{2,2′,2″,2‴-{{4′-{4‴-[(4,6-Dichloro-1,3,5-triazin-2-yl)amino]phenyl}-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetato)}europium(III) (**77**). UV (H_2O): 335, 296, 235.

{2,2′,2″,2‴-{{4′-{4‴-[(4,6-Dichloro-1,3,5-triazin-2-yl)amino]phenyl}-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetato)}terbium(III) (**78**). UV (H_2O): 327, 297, 235.

{2,2′,2″,2‴-{{4′-{3‴-[(4,6-Dichloro-1,3,5-triazin-2-yl)amino]phenyl}-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetato)}europium(III) (**79**). UV (H_2O): 331, 294, 238 (sh).

{2,2′,2″,2‴-{{4′-{3‴-[(4,6-Dichloro-1,3,5-triazin-2-yl)amino]phenyl}-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetato)}terbium(III) (**80**). UV (H_2O): 336, 298, 242.

{2,2′,2″,2‴-{{4′-{4‴-{3⁗-[(4,6-Dichloro-1,3,5-triazin-2-yl)amino]benzyloxy}phenyl}-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetato)}europium(III) (**81**). UV (H_2O): 330 (sh), 295, 236.

{2,2′,2″,2‴-{{4′-{4‴-{3⁗-[(4,6-Dichloro-1,3,5-triazin-2-yl)amino]benzyloxy}phenyl}-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetato)}terbium(III) (**82**). UV (H_2O): 330 (sh), 295, 234.

{2,2′,2″,2‴-{{4′-{3‴-{3⁗-[(4,6-Dichloro-1,3,5-triazin-2-yl)amino]benzyloxy}phenyl}-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetato)}europium(III) (**83**). UV (H_2O): 330, 295, 288, 274 (sh).

{2,2′,2″,2‴-{4′-{3‴-{3⁗-[(4,6-Dichloro-1,3,5-triazin-2-yl)amino]benzyloxy}phenyl}-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetato)}terbium(III) (**84**). UV (H_2O): 328, 295, 288, 240 (sh).

{2,2′,2″,2‴-{{4′-{2‴-{4⁗-[(4,6-Dichloro-1,3,5-triazin-2-yl)amino]phenyl}ethyl}-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetato)}europium(III) (**85**). UV (H_2O): 337, 325, 294, 284, 275, 238.

{2,2′,2″,2‴-{{4′-{2‴-{4⁗-[(4,6-Dichloro-1,3,5-triazin-2-yl)amino]phenyl}ethyl}-2,2′:6′,2″-terpyridine-6,6″-diyl}bis(methylenenitrilo)}tetrakis(acetato)}terbium(III) (**86**). UV (H_2O): 336, 324, 294, 285, 276, 238.

15. *Concentration Measurements.* The measurement of total lanthanide-ion (Eu^{3+} and Tb^{3+}) concentrations before, during, and after labelling were performed using a dissociative fluorescence enhancement system [2] based on the *Wallac-Delfia* enhancement soln. composed of 15 μM 4,4,4-trifluoro-1-(naphth-2-yl)butane-1,3-dione, 50 μM trioctylphosphine oxide, and 0.1% *Triton X-100* in acetate-phthalate buffer, pH 3.2.

16. *Coupling of the Chelates* **67**–**86** *to Protein.* The activated chelates were coupled to a model protein (rabbit anti-mouse IgG, *Dako*, Denmark). The isothiocyanato chelates **67**–**76** were incubated with IgG (1 mg) in carbonate buffer (500 μl, pH 9.3) overnight using a 100-fold molar ratio reagent/protein. The molar ratio reagent/protein in the case of the (4,6-dichloro-1,3,5-triazin-2-yl)amino chelates **77**–**86** was 20-fold. After the coupling reaction, the proteins were purified on a combined column of *Sephadex G50* (10 cm) and *Sepharose 6B* (30 cm) by eluting with 50 mM *Tris*-HCl buffer containing 0.15M NaCl and 0.05% NaN_3 soln. The fractions of corresponding labelled monomeric IgG were collected. The chelate concentrations in the protein fractions were measured from both the absorptions of conjugated chelates at 330 nm and the total lanthanide-ion concentrations by the dissociative fluorescence enhancement system (the time required for total ion dissociation from protein-bound chelate varied from a few h to 2 weeks). The purified protein conjugates and the labelling ratios (chelates per protein) were quantitated by calculating the protein yield or by measuring the absorbance at 280 nm and subtracting the absorption caused by the added chelates.

Luminescence Measurements. The luminescence parameters for free chelates were analyzed in borate buffer, pH 8.5. The ligand concentration was kept at 10 μM, and the lanthanide-ion concentrations varied between 0.1 and 1 μM depending on the luminescence intensities. The measurements were standardized using 0.1 μM Eu^{3+} in the *Wallac Delfia* enhancement soln. (molar absorptivity 37600, quantum yield 70% and luminescence yield 26320 [28]). The emission intensities of the lanthanide chelates were measured using the most intense emission line, at *ca.* 545 nm for Tb^{III} and 613 nm for Eu^{III}, constituting more than 80% of the total emission produced. The Tb^{III} luminescence was corrected for photomultiplier quantum-yield difference (1.39-fold at 545 as compared to the value at 613 nm). The luminescence parameters for chelate-labelled antibodies were measured similarly using appropriate dilutions of the conjugated proteins analyzed above. Results: *Tables 1* and *2*.

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