

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

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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-

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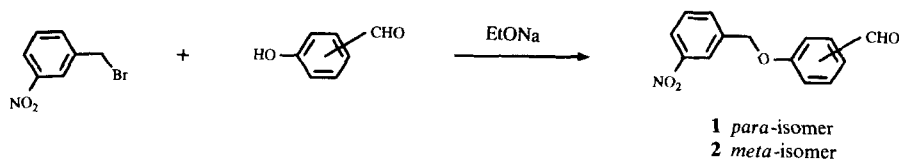
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hydroxybenzoic acid [6], 2,2',2'',2'''-{[4-(4-isothiocyanato-2-methoxyphenyl)pyridine-2,6-diyl]bis(methylenitrilo)}tetrakis(acetic acid) [7], (bipy.bipy.bipy)-cryptate [8], 2,2',2'',2'''-{[4-(4'-isothiocyanatophenylethynyl)pyridine-2,6-diyl]bis(methylenitrilo)}tetrakis(acetic acid) [9], and 2,2',2'',2'''-{[1-hydroxy-4-(3'-isothiocyanatobenzoyl)benzene-2,6-diyl]bis(methylenitrilo)}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.

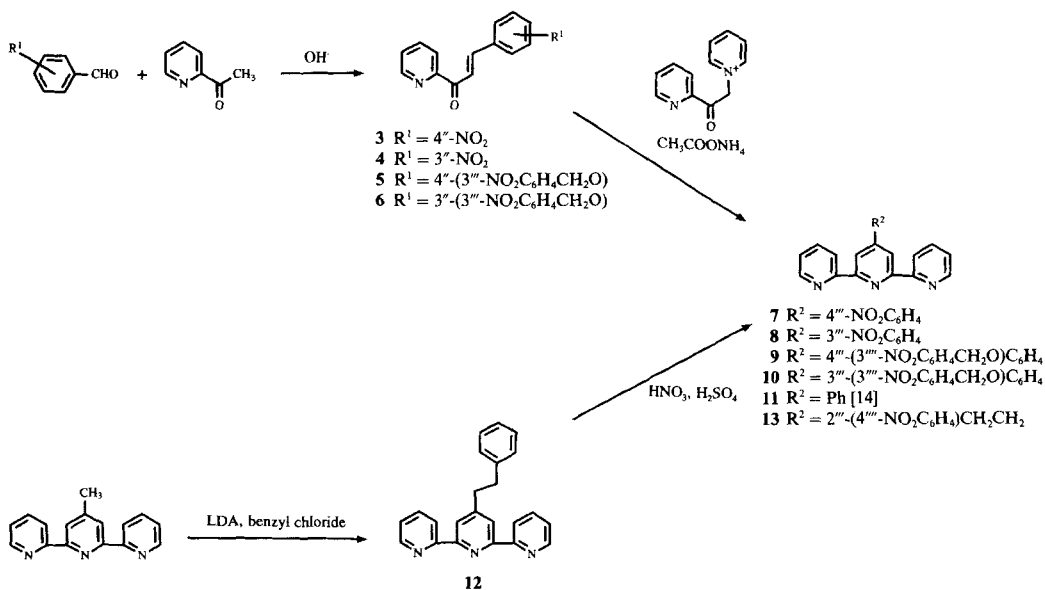
The Eu^{III} and Tb^{III} chelates of 2,2':6',2''-terpyridines and 2,2'-bipyridines were shown to be potential probe alternatives in time-resolved luminometric bioaffinity assays [11] [12]. In this article, we report the synthesis and luminescence properties of 20 different chelates composed of 2,2':6',2''-terpyridine as the energy-absorbing and donating group, Eu^{III} and Tb^{III} as the emitting ions, methylenitrilo(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



Scheme 2



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 AcONH₄ 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*-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 BH₃·THF. Without further purification, the formed bis(aminomethyl) compounds were carboxymethylated with alkyl bromoacetate to tetraesters **26–31**. Et(*i*-Pr)₂N was preferred to K₂CO₃ 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 NO₂ group of **21** with BH₃·THF (→ **32**, see below) was quite surprising because the NO₂ group of compounds **22–25** tolerated the CN-group reduction.

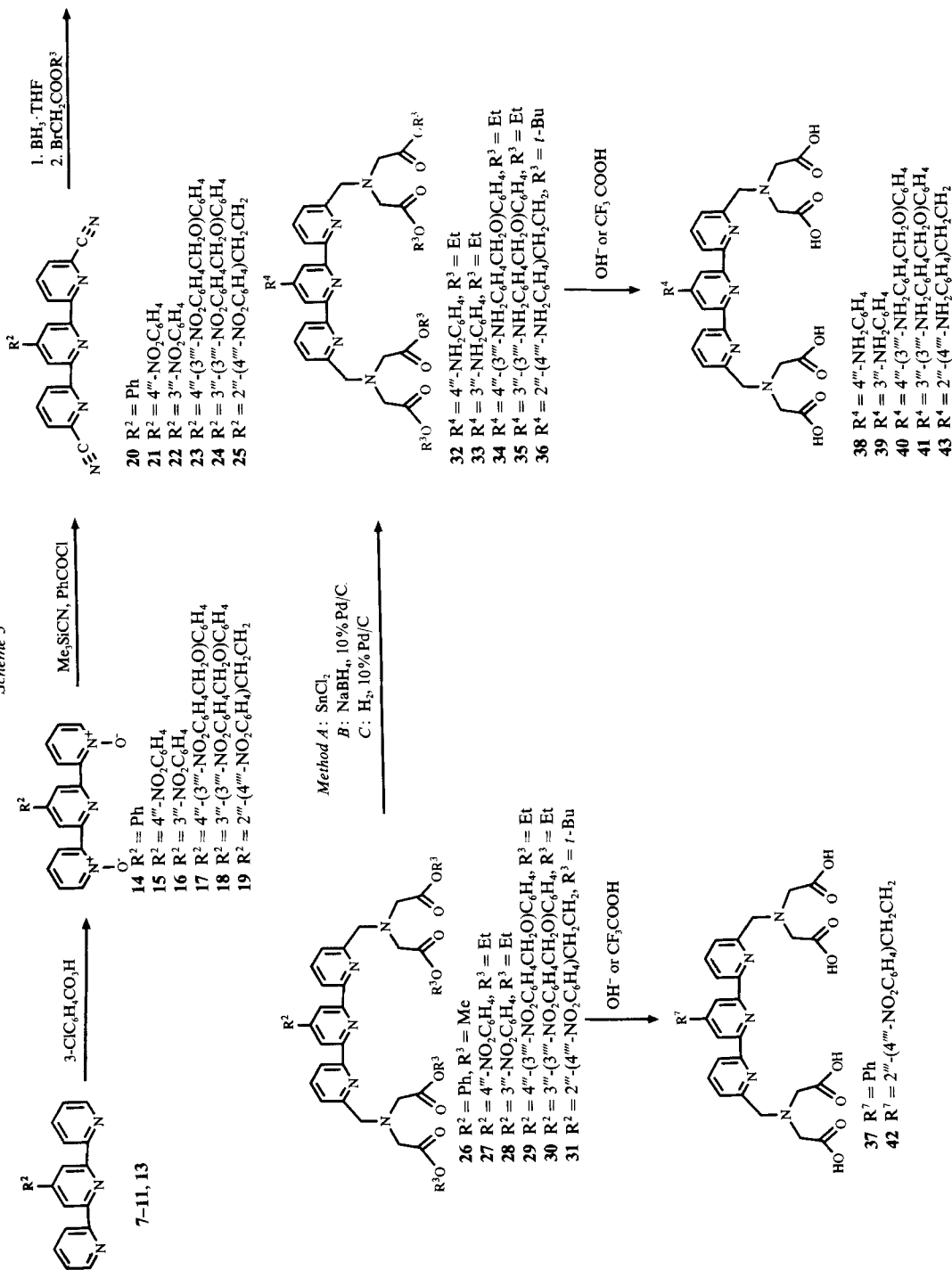
Three different methods were used to reduce the NO₂ group of **27–31** (*Scheme 3*). NaBH₄ with Pd/C was originally employed [19] [20]. However, the COOEt groups were often partly hydrolyzed under the reaction conditions. Later this method was substituted by the use of SnCl₂ in nonacidic abs. EtOH [21]. 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 SnCl₂ in abs. EtOH. The conventional use of SnCl₂ 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 NO₂ group was successfully performed with H₂ 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 CF₃COOH (→ **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/H₂SO₄ (→ **46**), and after reduction with NaBH₄ in EtOH, diol **47** was transformed with PBr₃ 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].

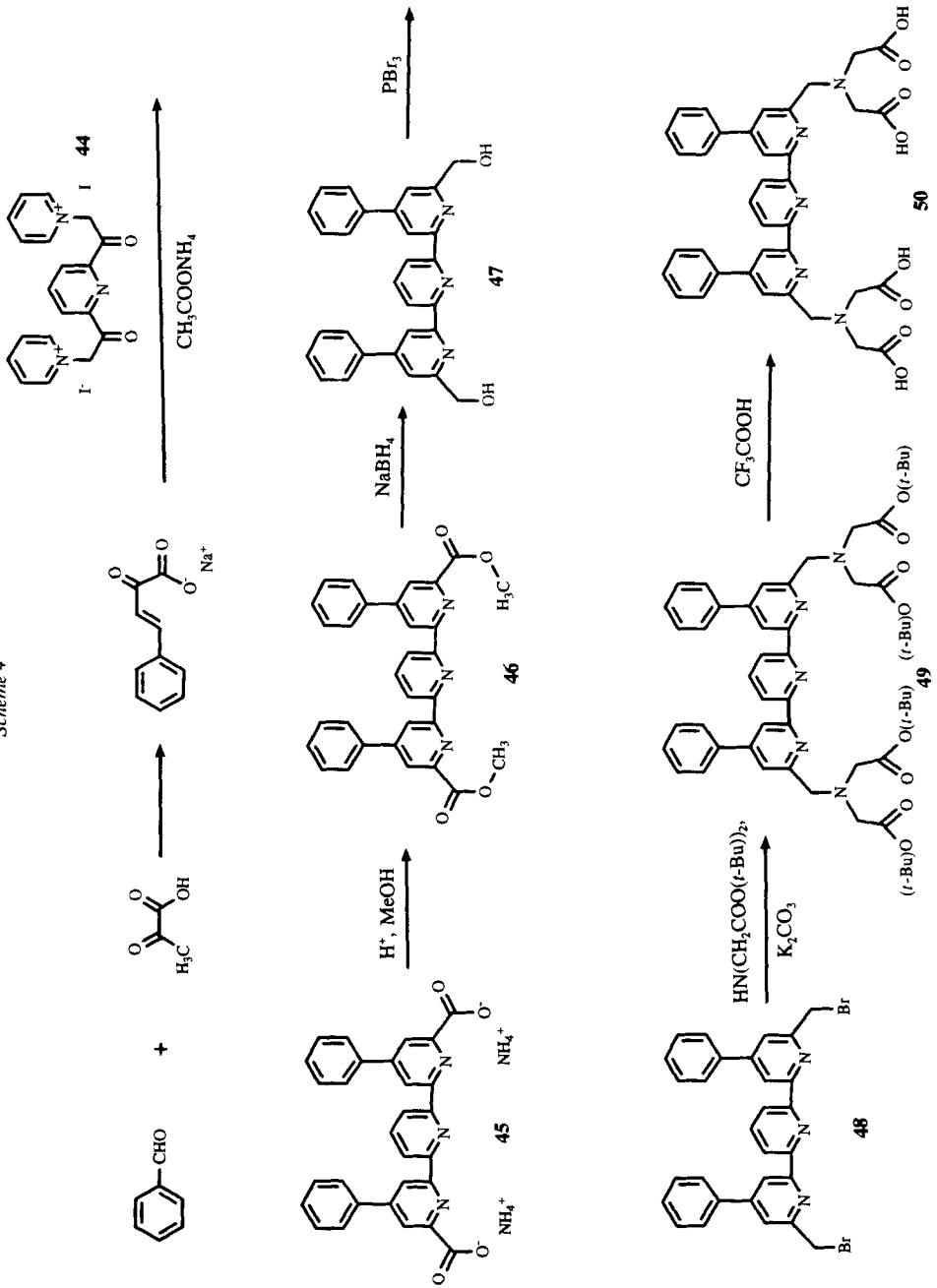
The lanthanide chelates **51–66** were prepared by stirring the tetrakis(acetic acids) **37–43** and **50** and LnCl₃ in slightly acidic solution and precipitating the excess lanthanides as Ln(OH)₃ by adjusting the pH to 8.5 (*Scheme 5*). The chelates were precipitated by the addition of acetone.

The activation of the NH₂ group of the chelates by their transformation to the isothiocyanato group (→ **67–76**) was performed with thiophosgene in H₂O/CHCl₃ by a

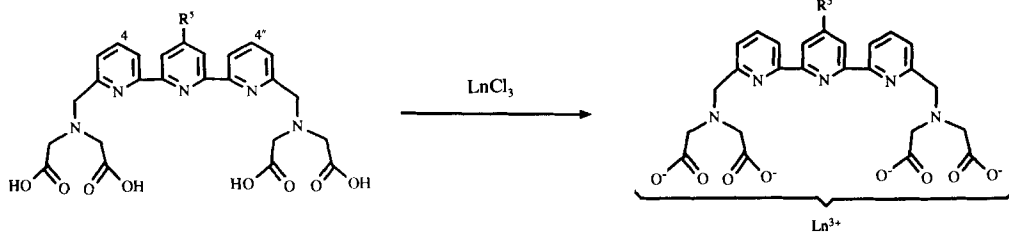
Scheme 3



Scheme 4

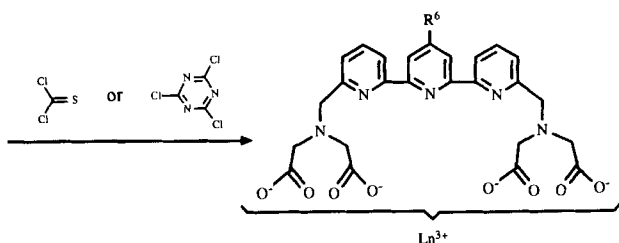


Scheme 5



37–43 $R^5 = R^4$ or R^7 (see Scheme 3)
 50 $R^5 = \text{H}$, instead 4-Ph and 4'-Ph

- 51 $R^5 = \text{Ph}$, Ln = Eu
 52 $R^5 = \text{Ph}$, Ln = Tb
 53 $R^5 = \text{H}$, instead 4-Ph and 4'-Ph, Ln = Eu
 54 $R^5 = \text{H}$, instead 4-Ph and 4'-Ph, Ln = Tb
 55 $R^5 = 4''\text{-NH}_2\text{C}_6\text{H}_4$, Ln = Eu
 56 $R^5 = 4''\text{-NH}_2\text{C}_6\text{H}_4$, Ln = Tb
 57 $R^5 = 3''\text{-NH}_2\text{C}_6\text{H}_4$, Ln = Eu
 58 $R^5 = 3''\text{-NH}_2\text{C}_6\text{H}_4$, Ln = Tb
 59 $R^5 = 4''\text{-}(3'''\text{-NH}_2\text{C}_6\text{H}_4\text{CH}_2\text{O})\text{C}_6\text{H}_4$, Ln = Eu
 60 $R^5 = 4''\text{-}(3'''\text{-NH}_2\text{C}_6\text{H}_4\text{CH}_2\text{O})\text{C}_6\text{H}_4$, Ln = Tb
 61 $R^5 = 3''\text{-}(3'''\text{-NH}_2\text{C}_6\text{H}_4\text{CH}_2\text{O})\text{C}_6\text{H}_4$, Ln = Eu
 62 $R^5 = 3''\text{-}(3'''\text{-NH}_2\text{C}_6\text{H}_4\text{CH}_2\text{O})\text{C}_6\text{H}_4$, Ln = Tb
 63 $R^5 = 2''\text{-}(4'''\text{-NO}_2\text{C}_6\text{H}_4)\text{CH}_2\text{CH}_2$, Ln = Eu
 64 $R^5 = 2''\text{-}(4'''\text{-NO}_2\text{C}_6\text{H}_4)\text{CH}_2\text{CH}_2$, Ln = Tb
 65 $R^5 = 2''\text{-}(4'''\text{-NH}_2\text{C}_6\text{H}_4)\text{CH}_2\text{CH}_2$, Ln = Eu
 66 $R^5 = 2''\text{-}(4'''\text{-NH}_2\text{C}_6\text{H}_4)\text{CH}_2\text{CH}_2$, Ln = Tb



- 67 $R^6 = 4''\text{-SCNC}_6\text{H}_4$, Ln = Eu
 68 $R^6 = 4''\text{-SCNC}_6\text{H}_4$, Ln = Tb
 69 $R^6 = 3''\text{-SCNC}_6\text{H}_4$, Ln = Eu
 70 $R^6 = 3''\text{-SCNC}_6\text{H}_4$, Ln = Tb
 71 $R^6 = 4''\text{-}(3'''\text{-SCNC}_6\text{H}_4\text{CH}_2\text{O})\text{C}_6\text{H}_4$, Ln = Eu
 72 $R^6 = 4''\text{-}(3'''\text{-SCNC}_6\text{H}_4\text{CH}_2\text{O})\text{C}_6\text{H}_4$, Ln = Tb
 73 $R^6 = 3''\text{-}(3'''\text{-SCNC}_6\text{H}_4\text{CH}_2\text{O})\text{C}_6\text{H}_4$, Ln = Eu
 74 $R^6 = 3''\text{-}(3'''\text{-SCNC}_6\text{H}_4\text{CH}_2\text{O})\text{C}_6\text{H}_4$, Ln = Tb
 75 $R^6 = 2''\text{-}(4'''\text{-SCNC}_6\text{H}_4)\text{CH}_2\text{CH}_2$, Ln = Eu
 76 $R^6 = 2''\text{-}(4'''\text{-SCNC}_6\text{H}_4)\text{CH}_2\text{CH}_2$, Ln = Tb
 77 $R^6 = 4''\text{-}[(\text{Cl}_2\text{C}_3\text{N}_3)\text{NH}]\text{C}_6\text{H}_4$, Ln = Eu
 78 $R^6 = 4''\text{-}[(\text{Cl}_2\text{C}_3\text{N}_3)\text{NH}]\text{C}_6\text{H}_4$, Ln = Tb
 79 $R^6 = 3''\text{-}[(\text{Cl}_2\text{C}_3\text{N}_3)\text{NH}]\text{C}_6\text{H}_4$, Ln = Eu
 80 $R^6 = 3''\text{-}[(\text{Cl}_2\text{C}_3\text{N}_3)\text{NH}]\text{C}_6\text{H}_4$, Ln = Tb
 81 $R^6 = 4''\text{-}\{3'''\text{-}[(\text{Cl}_2\text{C}_3\text{N}_3)\text{NH}]\text{C}_6\text{H}_4\text{CH}_2\text{O}\}\text{C}_6\text{H}_4$, Ln = Eu
 82 $R^6 = 4''\text{-}\{3'''\text{-}[(\text{Cl}_2\text{C}_3\text{N}_3)\text{NH}]\text{C}_6\text{H}_4\text{CH}_2\text{O}\}\text{C}_6\text{H}_4$, Ln = Tb
 83 $R^6 = 3''\text{-}\{3'''\text{-}[(\text{Cl}_2\text{C}_3\text{N}_3)\text{NH}]\text{C}_6\text{H}_4\text{CH}_2\text{O}\}\text{C}_6\text{H}_4$, Ln = Eu
 84 $R^6 = 3''\text{-}\{3'''\text{-}[(\text{Cl}_2\text{C}_3\text{N}_3)\text{NH}]\text{C}_6\text{H}_4\text{CH}_2\text{O}\}\text{C}_6\text{H}_4$, Ln = Tb
 85 $R^6 = 2''\text{-}\{4'''\text{-}[(\text{Cl}_2\text{C}_3\text{N}_3)\text{NH}]\text{C}_6\text{H}_4\}\text{CH}_2\text{CH}_2$, Ln = Eu
 86 $R^6 = 2''\text{-}\{4'''\text{-}[(\text{Cl}_2\text{C}_3\text{N}_3)\text{NH}]\text{C}_6\text{H}_4\}\text{CH}_2\text{CH}_2$, 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':6',2''-terpyridine-6,6''-diyl)bis(methylenenitrilo)]tetrakis(acetic acid) (see 51, $R^5 = \text{H}$) 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 ($\epsilon \cdot \Phi$) of the Europium(III) and Terbium(III) Chelates of 2,2',2'',2'''-2'''-[(2,2':6,2''-Terpyridine-6,6''-diyl)bis(methylenemitrilo)]tetrakis(acetic Acid) Derivatives

	Eu ³⁺			Tb ³⁺		
	λ_{exc} [nm]	λ [μ s]	$\epsilon \cdot \Phi$	λ_{exc} [nm]	τ [μ s]	$\epsilon \cdot \Phi$
<i>Uncoupled chelates</i>						
Parent compound (see 51 and 52 with R ⁵ = H) ^{a)}			2100	333	1100	3800
4'-Phenyl (51 and 52)	334	1310	2100	333	1100	3800
4,4''-Diphenyl (53 and 54)	293	1210	1970	293	530	1900
4'-[2'''-(4'''-Nitrophenyl)ethyl] (63 and 64)	339	1300	3900	339	260	1500
4'-[2'''-(4'''-Aminophenyl)ethyl] (65 and 66)	332	1340	580	270	577	32
	331	1240	220	332	697	53
<i>Chelates coupled to proteins</i>						
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'''-Isothiocyanatobenzoyloxy)phenyl] (71 and 72)	340	1520	2500	334	50	190
4'-[4'''-[(3'''-[(4,6-Dichloro-1,3,5-triazin-2-yl)amino]benzoyloxy)phenyl] (81 and 82)	333	1450	1800	333	110	190
4'-[3'''-(3'''-Isothiocyanatobenzoyloxy)phenyl] (73 and 74)	297	1200	790	300	30	35
4'-[3'''-[(3'''-[(4,6-Dichloro-1,3,5-triazin-2-yl)amino]benzoyloxy)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].

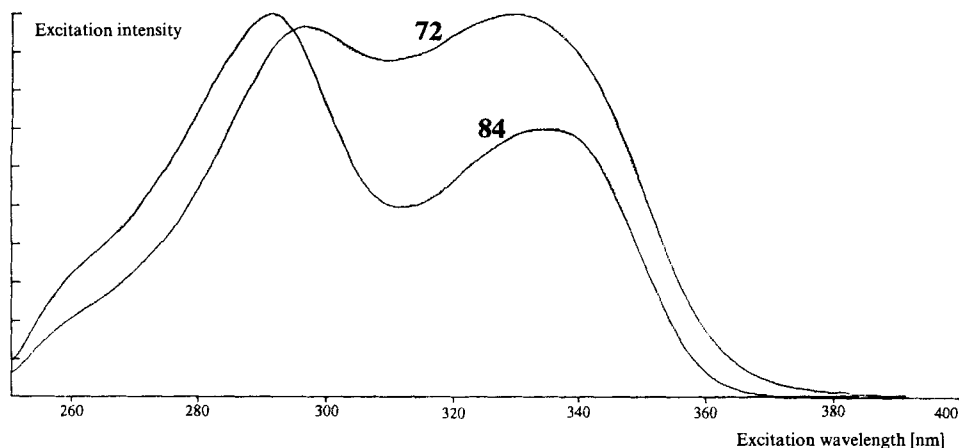


Figure. The excitation spectra of chelates **72** and **84**

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 R³ = 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 NO₂- and NH₂-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 H₂O 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 H₂O and D₂O [25]. As shown in *Table 2*, the effect of H₂O 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 H₂O, D₂O, and Protein Surface on Luminescence Yields ($\epsilon \cdot \Phi$) and Decay Times (τ) of Terbium(III) Chelates **72** and **82**

Chelate	<i>Tris</i> conjugate				Protein conjugate	
	($\epsilon \cdot \Phi$) _{H₂O}	($\epsilon \cdot \Phi$) _{D₂O}	τ _{H₂O}	τ _{D₂O}	($\epsilon \cdot \Phi$) _{H₂O}	τ _{H₂O}
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 μ s 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*. ¹H-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 CHCl₃ (100 ml), washed with 1M NaOH (50 ml) and H₂O (50 ml), dried (Na₂SO₄), and evaporated.

4-(3'-Nitrobenzyloxy)benzaldehyde (**1**). Yield: 99%. UV (EtOH): 268. IR (KBr): 1697 (C=O), 1529 (NO₂), 1352 (NO₂), 1267 (C—O—C). ¹H-NMR (CDCl₃): 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 (NO₂), 1348 (NO₂), 1263 (C—O—C). ¹H-NMR (CDCl₃): 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 H₂O (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 (NO₂), 1349 (NO₂). ¹H-NMR (CDCl₃): 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 (NO₂), 1343 (NO₂). ¹H-NMR (CDCl₃): 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 H₂O (10 ml). After stirring for 5 h at r.t., the mixture was evaporated and the residue triturated with H₂O (150 ml). The solid material was filtered and washed with H₂O 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 (NO₂), 1351 (NO₂), 1259 (C—O—C). ¹H-NMR (CDCl₃): 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 (NO₂), 1352 (NO₂), 1274 (C—O—C). ¹H-NMR (CDCl₃): 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 AcONH₄ (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 (NO₂), 1352 (NO₂). ¹H-NMR ((D₆)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 (NO₂), 1348 (NO₂). ¹H-NMR ((D₆)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 (NO₂), 1350 (NO₂), 1255 (C–O–C). ¹H-NMR (CDCl₃): 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 (NO₂), 1350 (NO₂), 1263 (C–O–C). ¹H-NMR (CDCl₃): 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-Pr)₂NH (0.85 g, 8.4 mmol) in dry THF (5 ml) was cooled to –70° and deaerated with N₂. 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. NH₄Cl soln. (30 ml), the aq. phase extracted with CH₂Cl₂ (2 × 10 ml) and the combined org. phase dried (Na₂SO₄) and evaporated: 2.35 g (94%). UV (EtOH): 279, 238. IR (film): 1601, 1584, 1558 (arom. C–C). ¹H-NMR (CDCl₃): 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. HNO₃ 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 H₂SO₄ (< 10°) and the mixture heated to 50° for 10 min, poured into ice-water, and neutralized with solid Na₂CO₃. After extraction with CHCl₃ (3 × 25 ml), the org. fractions were dried (Na₂SO₄) and evaporated. Yield: 84% (sample contained ca. 10% of 2''-nitrophenyl derivative). UV (EtOH): 278, 239. IR (film): 1518 (NO₂), 1345 (NO₂). ¹H-NMR (CDCl₃): 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 CH₂Cl₂ (80 ml). After stirring overnight, the mixture was washed with 10% Na₂CO₃ soln. (2 × 50 ml) and H₂O (50 ml), dried (Na₂SO₄), 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). ¹H-NMR (CDCl₃): 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 (NO₂), 1351 (NO₂), 1226 (N→O). ¹H-NMR ((D₆)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/CHCl₃. Yield: 89%. UV (EtOH): 280 (sh), 248. IR (film): 1532 (NO₂), 1350 (NO₂), 1240 (N→O). ¹H-NMR ((D₆)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 (NO₂), 1350 (NO₂), 1274 (C–O–C), 1248 (N→O). ¹H-NMR ((D₆)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 (NO₂), 1350 (NO₂), 1276 (C–O–C), 1248 (N→O).

¹H-NMR (CDCl₃): 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 CHCl₃). Yield: 53%. UV (EtOH): 275, 244. IR (film): 1516 (NO₂), 1344 (NO₂), 1263 (N→O). ¹H-NMR (CDCl₃): 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.* Me₃SiCN (5.46 g, 55 mmol) was added to **14** or **15–19** (5.5 mmol) in CH₂Cl₂ (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% K₂CO₃ soln. (160 ml) added, the mixture stirred for 15 min, and the precipitate filtered and washed with H₂O and cold CH₂Cl₂.

4'-Phenyl-2,2':6',2''-terpyridine-6,6''-dicarbonitrile (20). Yield: 82%. UV (EtOH): 281, 258. IR (film): 2239 (C≡N). ¹H-NMR (CDCl₃): 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 (NO₂), 1352 (NO₂). ¹H-NMR ((D₆)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 (NO₂), 1359 (NO₂). ¹H-NMR ((D₆)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 (NO₂), 1350 (NO₂), 1264 (C–O–C). ¹H-NMR (CDCl₃): 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 (NO₂), 1352 (NO₂), 1273 (C–O–C). ¹H-NMR ((D₆)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 K₂CO₃, the aq. phase was extracted with CHCl₃ (2 × 25 ml), the combined org. phase dried (Na₂SO₄) and evaporated, and the residue crystallized from MeCN/THF. Yield: 69%. UV (EtOH): 288, 258. IR (film): 2237 (C≡N), 1516 (NO₂), 1345 (NO₂). ¹H-NMR (CDCl₃): 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 N₂. BH₃·THF (1M; 25 ml, 25.0 mmol) was added within 10 min. After stirring overnight, excess BH₃ was destroyed 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), BrCH₂COOEt (1.44 g, 7.4 mmol; BrCH₂COOMe or BrCH₂COO(*t*-Bu) for **26** and **31**, resp.), dry (i-Pr)₂EtN (3.59 g, 28 mmol), and dry MeCN (15 ml) was refluxed for 20 h. After evaporation, the residue was dissolved in CHCl₃ (40 ml) the soln. washed with H₂O (3 × 20 ml) and dried (Na₂SO₄), and the product purified by FC (silica gel).

Tetramethyl 2,2',2'',2'''-[4'-(4'''-Nitrophenyl)-2,2':6',2''-terpyridine-6,6''-diyl]bis(methylenenitrilo)}tetrakis(acetate) (26). FC: 2% MeOH in CHCl₃. Yield: 36%. UV (EtOH): 281, 253. IR (film): 1747 (C=O), 1201 (C–O). ¹H-NMR (CDCl₃): 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/Et₃N 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 (NO₂), 1348 (NO₂), 1193 (C–O). ¹H-NMR (CDCl₃): 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 CHCl₃. Yield: 16%. UV (EtOH): 282, 247. IR (film): 1744 (C=O), 1532 (NO₂), 1349 (NO₂), 1193 (C–O). ¹H-NMR (CDCl₃): 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 CHCl₃. Yield: 57%. UV (EtOH): 288, 257. IR (film): 1738 (C=O), 1732 (C=O), 1532 (NO₂), 1350 (NO₂), 1246 (C–O–C), 1193 (C–O). ¹H-NMR (CDCl₃): 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 CHCl₃. Yield: 38%. UV (EtOH): 280, 254. IR (film): 1744 (C=O), 1532 (NO₂), 1350 (NO₂), 1265 (C–O–C), 1193 (C–O). ¹H-NMR (CDCl₃): 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/Et₃N 5:1:1. Yield: 40%. UV (EtOH): 282, 242. IR (film): 1738 (C=O), 1522 (NO₂), 1346 (NO₂), 1144 (C–O). ¹H-NMR (CDCl₃): 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.* SnCl₂·2H₂O (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 H₂O (25 ml). The mixture was neutralized with sat. NaHCO₃ soln., the aq. phase extracted with CHCl₃ (3 × 20 ml), and the combined org. phase dried (Na₂SO₄).

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 NaBH₄ (4 mg, 0.1 mmol). After 1 h stirring, the mixture was filtered, the filtrate evaporated, the residue dissolved in H₂O, and the product extracted with CHCl₃. The CHCl₃ phase was dried (Na₂SO₄).

Method C. A mixture of nitro compound (0.48 mmol), 10% Pd/C (50 mg), and MeOH (20 ml) was stirred under H₂ (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 (CHCl₃): 1742 (C=O), 1194 (C–O). ¹H-NMR (CDCl₃): 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/Et₃N 5:3:2). Yield: 100% (79% with *Method B*). UV (EtOH): 315 (sh), 280, 248. IR (film): 1743 (C=O), 1196 (C–O). ¹H-NMR ((D₆)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 CHCl₃). Yield: 69%. UV (EtOH): 335 (sh), 294, 233. IR (film): 1738 (C=O), 1730 (C=O), 1194 (C–O). ¹H-NMR (CDCl₃): 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 CHCl₃). Yield: 64%. UV (EtOH): 288, 241. IR (film): 1738 (C=O), 1732 (C=O), 1195 (C–O). ¹H-NMR ((D₆)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/Et₃N 5:1:1). Yield: 48%. UV (EtOH): 286, 241. IR (film): 1738 (C=O), 1733 (C=O), 1143 (C–O). ¹H-NMR

(CDCl₃): 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, 0.5 M KOH/EtOH (6 ml), and H₂O (1 ml) was stirred for 2 h at r.t. The mixture was neutralized with 1 M 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 CF₃COOH (5 ml) was stirred for 2 h at r.t. After evaporation, the mixture was triturated with Et₂O and filtered.

2,2',2'',2'''-[4'-Phenyl-2,2':6',2''-terpyridine-6,6''-diyl]bis(methylenenitrilo) tetrakis(acetic Acid) (**37**). UV (H₂O): 280, 250. IR (KBr): 1728 (C=O), 1612 (C=O), 1399 (C–O), 1219 (C–O). ¹H-NMR ((D₆)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 (H₂O): 325, 292, 233. IR (KBr): 1728 (C=O), 1684 (C=O), 1625 (C=O), 1396 (C–O), 1199 (C–O). ¹H-NMR ((D₆)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 (H₂O): 325, 282, 245. IR (KBr): 1719 (C=O), 1623 (C=O), 1395 (C–O), 1214 (C–O). ¹H-NMR (D₂O): 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'''-Aminobenzoyloxy)phenyl]-2,2':6',2''-terpyridine-6,6''-diyl]bis(methylenenitrilo) tetrakis(acetic Acid) (**40**). UV (H₂O): 325 (sh), 291, 232. IR (KBr): 1627 (C=O), 1604 (C=O), 1398 (C–O), 1260 (C–O–C), 1187 (C–O). ¹H-NMR ((D₆)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'''-Aminobenzoyloxy)phenyl]-2,2':6',2''-terpyridine-6,6''-diyl]bis(methylenenitrilo) tetrakis(acetic Acid) (**41**). UV (H₂O): 315 (sh), 283, 240 (sh). IR (KBr): 1630 (C=O), 1607 (C=O), 1395 (C–O), 1268 (C–O–C), 1205 (C–O). ¹H-NMR ((D₆)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'''-Nitrophenyl)ethyl]-2,2':6',2''-terpyridine-6,6''-diyl]bis(methylenenitrilo) tetrakis(acetic Acid) (**42**). UV (H₂O): 284, 243. IR (KBr): 1732 (C=O), 1519 (NO₂), 1347 (NO₂), 1195 (C–O). ¹H-NMR ((D₆)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 (H₂O): 287, 242. IR (KBr): 1734 (C=O), 1199 (C–O). ¹H-NMR ((D₆)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), I₂ (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). ¹H-NMR ((D₆)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), AcONH₄ (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 (H₂O): 242. IR (KBr): 1577, 1406 (COO). ¹H-NMR ((D₆)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 2 M NaOH (400 ml) and the pH adjusted to 1 with 6 M HCl. The diacid was filtered and washed with H₂O. A mixture of dry diacid (0.77 g, 1.6 mmol), MeOH (10 ml), and conc. H₂SO₄ soln. (0.05 ml) was refluxed for 24 h. The cold mixture was filtered and the product crystallized from CH₂Cl₂: 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). ¹H-NMR (CDCl₃): 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**). NaBH₄ (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. NaHCO₃ soln. (5 ml) added, and the soln. brought to boiling. H₂O (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). ¹H-NMR ((D₆)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 PBr₃ (0.12 g, 0.44 mmol) in CHCl₃ (1 ml) was added to a soln. of **47** (0.13 g, 0.29 mmol) in CHCl₃ (17 ml). The mixture was refluxed for 11 h, whereafter the mixture was neutralized with 5% NaHCO₃ soln. The aq. layer was extracted with CHCl₃ (3 × 20 ml), the combined org. phase dried (Na₂SO₄) and evaporated, and the residue crystallized from CH₂Cl₂: 48 mg (29%). M.p. 253.5–255.5°. UV (EtOH): 303, 249. IR (film): 1610, 1575, 1549 (arom. C–C). ¹H-NMR (CDCl₃): 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 K₂CO₃ (120 mg, 0.87 mmol), and dry MeCN (5 ml) was refluxed overnight. The mixture was evaporated and the suspension of the residue in CHCl₃ (10 ml) washed with 5% NaHCO₃ soln. (2 × 5 ml) and H₂O (2 × 10 ml) and dried (Na₂SO₄): 47 mg (59%). UV (EtOH): 304, 247. ¹H-NMR (CDCl₃): 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 CF₃COOH (3 ml). CF₃COOH was evaporated, the residue triturated with Et₂O, and the product filtered: 35 mg (100%). UV (H₂O): 310, 247. IR (KBr): 1734 (C=O), 1395 (C–O), 1200 (C–O). ¹H-NMR ((D₆)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 H₂O (2 ml) and the pH adjusted to 6.5 with solid NaHCO₃, EuCl₃ or TbCl₃ (0.16 mmol) in H₂O (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 (H₂O): 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 (H₂O): 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 (H₂O): 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 (H₂O): 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 (H₂O): 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 (H₂O): 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 (H₂O): 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 (H₂O): 335, 298, 240.

$\{2,2',2'',2'''-[4'-(4'''-(3'''-Aminobenzoyloxy)phenyl)-2,2':6',2''-terpyridine-6,6''-diyl]bis(methylenenitrilo) tetrakis(acetato)\}europium(III)$ (**59**). UV (H₂O): 330, 297, 235.

$\{2,2',2'',2'''-[4'-(4'''-(3'''-Aminobenzoyloxy)phenyl)-2,2':6',2''-terpyridine-6,6''-diyl]bis(methylenenitrilo) tetrakis(acetato)\}terbium(III)$ (**60**). UV (H₂O): 330, 296, 235.

$\{2,2',2'',2'''-[4'-(3'''-(3'''-Aminobenzoyloxy)phenyl)-2,2':6',2''-terpyridine-6,6''-diyl]bis(methylenenitrilo) tetrakis(acetato)\}europium(III)$ (**61**). UV (H₂O): 328, 294, 240 (sh).

$\{2,2',2'',2'''-[4'-(3'''-(3'''-Aminobenzoyloxy)phenyl)-2,2':6',2''-terpyridine-6,6''-diyl]bis(methylenenitrilo) tetrakis(acetato)\}terbium(III)$ (**62**). UV (H₂O): 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 (H₂O): 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 (H₂O): 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 (H₂O): 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 (H₂O): 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), NaHCO₃ (55 mg, 0.66 mmol), and CHCl₃ (3.5 ml). After stirring for 1 h, the H₂O phase was washed with CHCl₃ (3 × 2 ml), acetone was added to the aq. soln. and the precipitate filtered and washed with acetone.

$\{2,2',2'',2'''-\{4'-[4'''-(4''''-Isothiocyanatophenyl)-2,2':6',2''-terpyridine-6,6''-diyl]bis(methylenenitrilo)\}tetrakis(acetato)\}europium(III)$ (67). UV (H₂O): 330, 297, 234. IR (KBr): 2062 (S=C=N).

$\{2,2',2'',2'''-\{4'-[4'''-(4''''-Isothiocyanatophenyl)-2,2':6',2''-terpyridine-6,6''-diyl]bis(methylenenitrilo)\}tetrakis(acetato)\}terbium(III)$ (68). UV (H₂O): 320 (sh), 297, 230. IR (KBr): 2082 (S=C=N).

$\{2,2',2'',2'''-\{4'-[3'''-(4''''-Isothiocyanatophenyl)-2,2':6',2''-terpyridine-6,6''-diyl]bis(methylenenitrilo)\}tetrakis(acetato)\}europium(III)$ (69). UV (H₂O): 332, 295, 286, 240. IR (KBr): 2115 (S=C=N).

$\{2,2',2'',2'''-\{4'-[3'''-(3''''-Isothiocyanatophenyl)-2,2':6',2''-terpyridine-6,6''-diyl]bis(methylenenitrilo)\}tetrakis(acetato)\}terbium(III)$ (70). UV (H₂O): 330, 294, 285, 240 (sh). IR (KBr): 2130 (S=C=N).

$\{2,2',2'',2'''-\{4'-[4'''-(3''''-Isothiocyanatobenzoyloxy)phenyl]-2,2':6',2''-terpyridine-6,6''-diyl\}bis(methylenenitrilo)\}tetrakis(acetato)\}europium(III)$ (71). UV (H₂O): 330, 296, 237 (sh). IR (KBr): 2107 (S=C=N).

$\{2,2',2'',2'''-\{4'-[4'''-(3''''-Isothiocyanatobenzoyloxy)phenyl]-2,2':6',2''-terpyridine-6,6''-diyl\}bis(methylenenitrilo)\}tetrakis(acetato)\}terbium(III)$ (72). UV (H₂O): 330 (sh), 296, 237. IR (KBr): 2113 (S=C=N).

$\{2,2',2'',2'''-\{4'-[3'''-(3''''-Isothiocyanatobenzoyloxy)phenyl]-2,2':6',2''-terpyridine-6,6''-diyl\}bis(methylenenitrilo)\}tetrakis(acetato)\}europium(III)$ (73). UV (H₂O): 328, 295, 286, 267 (sh). IR (KBr): 2118 (S=C=N).

$\{2,2',2'',2'''-\{4'-[3'''-(3''''-Isothiocyanatobenzoyloxy)phenyl]-2,2':6',2''-terpyridine-6,6''-diyl\}bis(methylenenitrilo)\}tetrakis(acetato)\}terbium(III)$ (74). UV (H₂O): 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 (H₂O): 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 (H₂O): 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 H₂O (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 (H₂O): 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 (H₂O): 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 (H₂O): 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 (H₂O): 336, 298, 242.

$\{2,2',2'',2'''-\{4'-[4'''-[3'''-[4,6-Dichloro-1,3,5-triazin-2-yl]amino]benzoyloxy]phenyl\}-2,2':6',2''-terpyridine-6,6''-diyl\}bis(methylenenitrilo)\}tetrakis(acetato)\}europium(III)$ (81). UV (H₂O): 330 (sh), 295, 236.

$\{2,2',2'',2'''-\{4'-[4'''-[3'''-[4,6-Dichloro-1,3,5-triazin-2-yl]amino]benzoyloxy]phenyl\}-2,2':6',2''-terpyridine-6,6''-diyl\}bis(methylenenitrilo)\}tetrakis(acetato)\}terbium(III)$ (82). UV (H₂O): 330 (sh), 295, 234.

$\{2,2',2'',2'''-\{4'-[3'''-[4,6-Dichloro-1,3,5-triazin-2-yl]amino]benzoyloxy]phenyl\}-2,2':6',2''-terpyridine-6,6''-diyl\}bis(methylenenitrilo)\}tetrakis(acetato)\}europium(III)$ (83). UV (H₂O): 330, 295, 288, 274 (sh).

$\{2,2',2'',2'''-\{4'-[3'''-[3'''-[4,6-Dichloro-1,3,5-triazin-2-yl]amino]benzoyloxy]phenyl\}-2,2':6',2''-terpyridine-6,6''-diyl\}bis(methylenenitrilo)\}tetrakis(acetato)\}terbium(III)$ (84). UV (H₂O): 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 (H₂O): 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 (H₂O): 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 $15\ \mu\text{M}$ 4,4-trifluoro-1-(naphth-2-yl)butane-1,3-dione, $50\ \mu\text{M}$ 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 ($500\ \mu\text{l}$, 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 $10\ \mu\text{M}$, and the lanthanide-ion concentrations varied between 0.1 and $1\ \mu\text{M}$ depending on the luminescence intensities. The measurements were standardized using $0.1\ \mu\text{M}$ 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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