Synthesis of Lanthanide(III) Chelates by Using 'Click' Chemistry

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The copper(I)-catalyzed dipolar [2 + 3] cycloaddition reaction of an azide and a terminal alkyne is exploited in the preparation of various europium(III), terbium(III), and dysprosium(III) chelates (*Schemes 1–3*). By changing the nature of the alkyne and the azide, a wide range of chelates and biomolecule-labeling reactants were obtained. The photophysical properties (*Table*) of the synthesized chelates are also discussed.

Introduction. – The copper(I)-catalyzed *Huisgen – Sharpless* dipolar [2 + 3] cycloaddition of azide and alkyne is a powerful direct method to prepare 1,4-disubstituted 1*H*-1,2,3-triazoles [1]. This reaction has been used increasingly in modular drug development, in the preparation of small-molecule radiopharmaceuticals, in DNA sequencing [2], and also for the conjugation of various label molecules to nanomaterials [3] and biomolecules [4]. The reaction has also been exploited in the preparation of peptidomimetics [5], oligonucleotide – carbohydrate conjugates [6], and various organic molecules [7].

The labels disclosed in the literature of 'click' chemistry are organic dyes. However, the organic fluorophores suffer from several drawbacks, such as *Raman* and *Rayleigh* scattering, low water solubility and concentration quenching. Thus, multilabeling of biomolecules with organic fluorophores may not enhance the detection sensitivity to the degree required in several applications. Furthermore, this type of labels may decrease the water solubility of the target molecule dramatically.

Lanthanide(III) chelates have several special properties which make them excellent alternatives in bioaffinity assays [8]. Their large *Stokes*' shift has a decreasing effect on scattering phenomena. The long fluorescence decay after excitation of these molecules allows time-delayed signal detection, which eliminates completely the background luminescence originating, *e.g.*, from buffer components, plastics, and biomaterials. The very narrow emission lines allow the use of effective filters which diminish the background. Since the lanthanide(III) chelates do not suffer from concentration quenching, it is possible to have several luminescent chelates in close proximity. Furthermore, the chelates are most commonly readily soluble in water. The different photophysical properties of europium, samarium, terbium, and dysprosium chelates enable even development of multiparametric homogeneous assays; with conventional fluorescent compounds, this is much more difficult because of the strong overlapping of the emission spectra.

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We show here the versatility of *Huisgen*'s cycloaddition reaction in the preparation of lanthanide(III) chelates and biomolecule-labeling reactants needed in bioanalytical applications. Also the photophysical properties of the chelates are discussed.

Results and Discussion. – *Synthesis of the Luminescent Lanthanide(III) Chelates.* Terbium(III) chelates based on (1*H*-pyrazole-1,3-diyl)bis(pyridine) subunits and europium(III) chelates based on terpyridine subunits have excellent photophysical properties including a high luminescent quantum yield and a relatively high energy of their lowest triplet states [9]. Indeed, several analogues, which allow biomolecule derivatization in solution and on solid phase have been synthesized and used in various bioanalytical assays based on time-resolved fluorescence (TRF) [10].

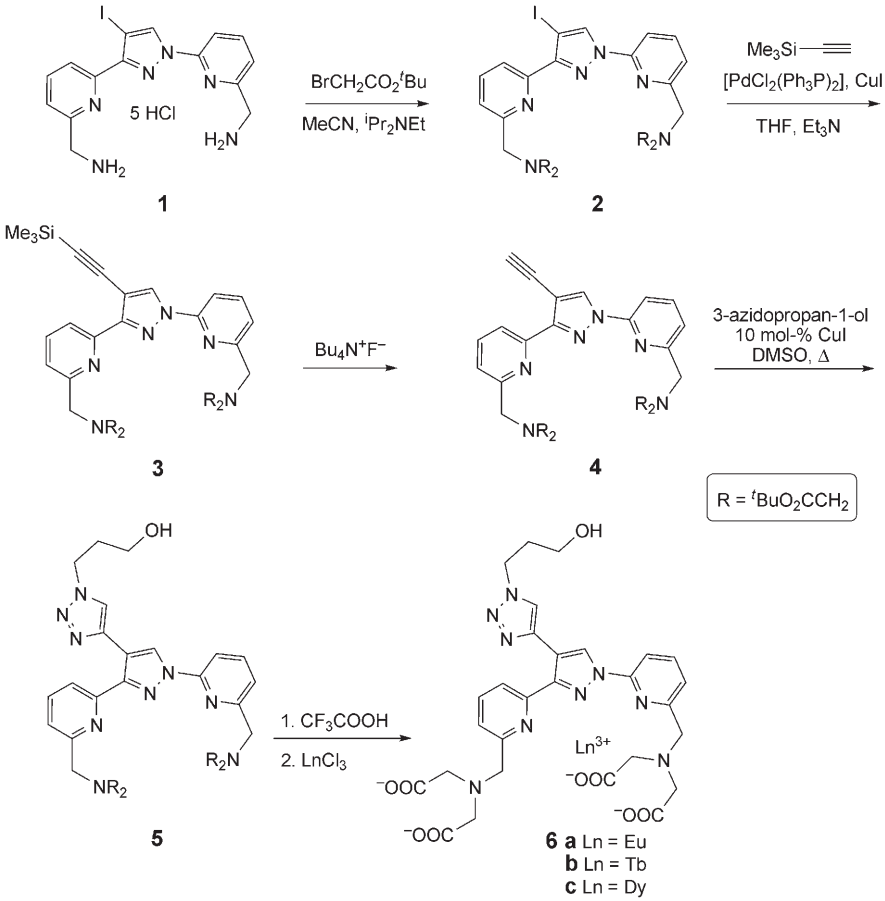
To investigate the applicability of 'click' chemistry for the preparation of lanthanide(III) chelates based on (1*H*-pyrazole-1,3-diyl)bis(pyridine) subunits, the iodo derivative **1** [11] was chosen as a starting material (*Scheme 1*). It was initially alkylated with *tert*-butyl bromoacetate in MeCN in the presence of $^{i}Pr_2NEt$ as the base. The product **2** was then converted to the corresponding ethynyl derivative **3** by *Sonogashira* reaction with ethynyltrimethylsilane. Removal of the silyl group with Bu_4NF yielded **4**. Reaction of **4** with 3-azidopropan-1-ol under the recently reported reaction conditions [7a] gave rise to the desired cycloaddition product **5** in 60% yield after purification by column chromatography (silica gel). However, 2 equiv. of the azide had to be used to complete the reaction. Furthermore, it was essential to wash the crude product with aqueous H_4edta solution to remove copper ions chelated to the ligand **5** (H_4edta = *N*,*N*′-ethane-1,2-diylbis[*N*-(carboxyethyl)glycine]. Removal of the *tert*-butyl groups by acidolysis followed by treatment with europium, terbium, or dysprosium chloride afforded the corresponding lanthanide(III) chelates **6a**–**c**.

The same synthetic strategy was used also in the preparation of the corresponding terpyridine derivative **11** starting from the bromo derivative **7** [12] (*via* the intermediates **8**–**10**; *Scheme 2*).

The Tethering Strategy. To investigate the versatility of the copper(I)-catalyzed cycloaddition reaction in the preparation of various lanthanide(III) chelates and biomolecule-labeling reactants, the model ligand **12** [12] was allowed to react with ω-substituted azides **13a**–**c** as shown in *Scheme 3*. Accordingly, by using the reaction conditions discussed above, ligands tethered to hydroxy, amino, and carboxylic acid functions, *i.e.*, **14a**–**c**, were obtained.

We have already reported a solid-phase method for the labeling of oligonucleotides [11][13], oligopeptides [14][15], and steroids [16]. The approach includes synthesis of oligonucleotide and oligopeptide building blocks which can be introduced to the biomolecule structure by means of oligonucleotide and oligopeptide synthesizers and phosphoramidite and Fmoc chemistry, respectively. Upon completion of the chain assembly, the oligomers are deprotected and finally treated with the appropriate lanthanide(III) citrate producing the desired biomolecule conjugates. The preparation of oligopeptide building blocks can be considerably simplified by means of 'click' chemistry: the reaction of the ligand **12** with azidopropanoic acid or the commercially available 4-azidophenylalanine derivative **13c** yielded **14b** and **14c**, blocks allowing mono- and multilabeling of oligopeptides, respectively.

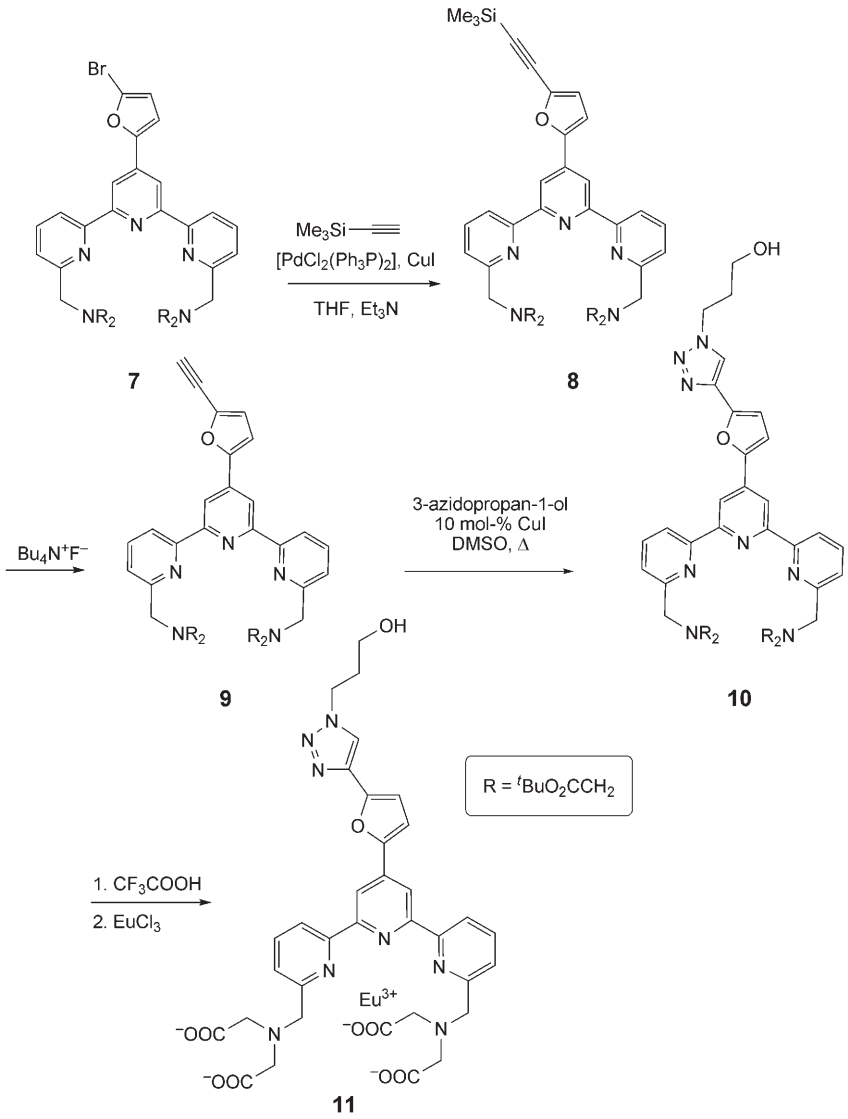
Scheme 1



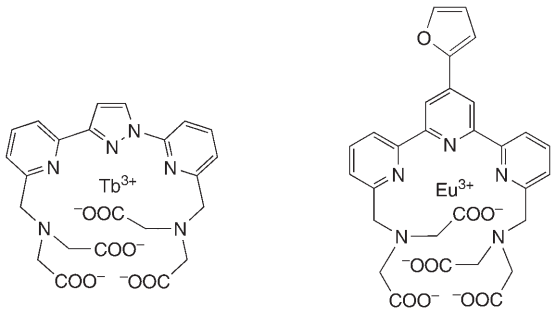
Photophysics. Stable luminescent lanthanide(III) chelates consist of a ligand with a reactive group for covalent conjugation to bioactive molecules, an aromatic structure which absorbs the excitation energy and transfers it to the lanthanide ion, and additional chelating groups such as carboxylic acid moieties and amines. Since the aromatic structure has a dramatic effect on the photophysical properties of the chelates, it was important to clarify the effect of the conjugated 1*H*-1,2,3-triazole subunit present in the chelates synthesized.

The measured excitation and emission wavelengths as well as the decay times (τ), luminescence yields ($\varepsilon\Phi$), and absorption maxima of the chelates **6a**–**c** and **11** are presented in the *Table*. Also the spectral properties of the previously synthesized chelates without the 1*H*-1,2,3-triazole subunit (*i.e.*, compounds **15** and **16**) are included for comparison. The luminescence intensities were determined by integrating the emissions from 450 to 750 nm.

Scheme 2

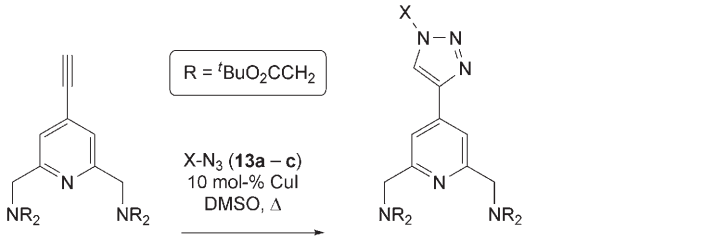


The observed emission spectra were typical for lanthanide chelates corresponding to the $^5D_0 \rightarrow {}^7F_j$, $^5D_4 \rightarrow {}^7F_j$, and $^4F_{9/2} \rightarrow {}^6H_j$ transitions for Eu^{3+}, Tb^{3+}, and Dy^{3+}, respectively. The main emission lines of chelates were centered as usual around 616 (Eu^{3+}), 544 (Tb^{3+}), and 574 nm (Dy^{3+}). A typical example of the excitation and emission spectra of a chelate is shown in the *Figure*. The 1*H*-1,2,3-triazole subunit did not have a significant effect on the photophysical properties of the terbium(III) chelates based on (1*H*-pyrazole-1,3-diyl)bis(pyridine) (see data of **6b** and **15**). In



15 **16**

Scheme 3



12

14 **a** X = $HOCH_2CH_2CH_2$
b X = $HOOCCH_2CH_2$
c X = 4-[HOOCCH(NHFmoc)CH_2]–C_6H_4

Table. *Photophysical Properties of the Synthesized Lanthanide(III) Chelates*[a]).

	λ_{exc}/nm	λ_{em}/nm (rel. int. %)	$\varepsilon\Phi$	τ/ms	λ_{max} (UV)/nm
6a	327	593 (14), 616 (50) 654 (4), 692 (32)	2310	1.03	321
6b	324	491 (21), 544 (55) 587 (15), 623 (8)	10000	2.61	323
6c	328	479 (39), 574 (56) 658 (2), 755 (2)	211	0.01	323
11	341	595 (13), 617 (50) 697 (37)	187	1.26	357
15	329	490 (21), 544 (55) 587 (15), 622 (8)	10580	2.41	327
16[b])	340	615	2100	1.08	336

[a]) The measurements were carried out in *Tris* buffer containing 0.9% NaCl at pH 7.75. The luminescence yields were measured by a relative method described in [9a]. [b]) Data from [12].

contrast, the addition of 1*H*-1,2,3-triazole on the terpyridine-based europium(III) chelate collapsed the luminescence yield (see data of **11** and **16**). This observed decrease in the luminescence yield is probably due to the increased conjugation reflected in the absorption maxima of the terpyridine derivative **11**. In other words, the large difference observed in the absorption and excitation maximum of **11** indicates that the ligand no longer transfers energy efficiently to the lanthanide ion. This phenomenom was not observed with the highly luminescent chelate **6b**. These observations indicate the importance of understanding of the effect of 1*H*-1,2,3-triazole on the photophysical properties of the chelate.

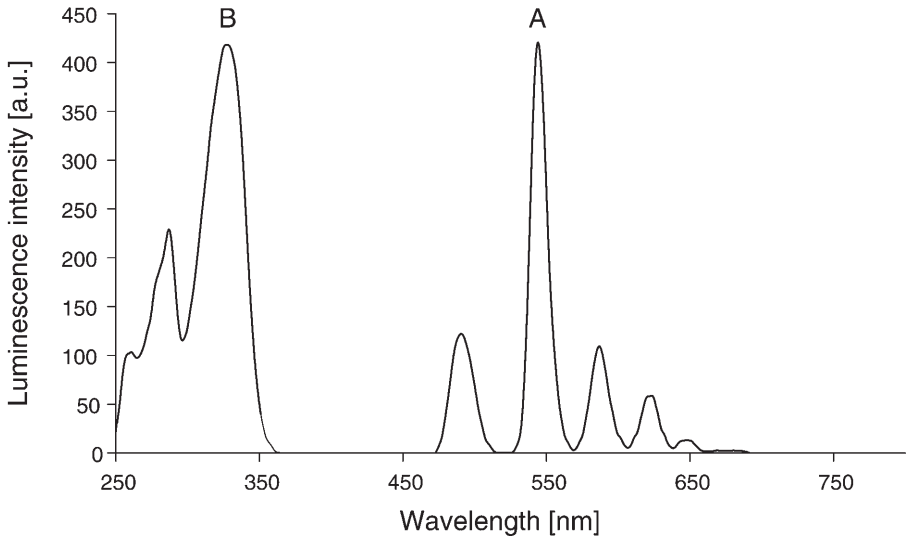


Figure. *Emission* (A) *and Excitation* (B) *Spectrum of Compound* **6b**

Conclusions. – Copper(I)-catalyzed [2 + 3] dipolar cycloaddition reactions are suitable for the preparation of lanthanide(III) chelates. By changing the structure of the chromophore and the tether group, a wide range of chelates and biomolecule-labeling reactants can be obtained. However, in some instances, the additional conjugation in the chromophore caused by the 1*H*-1,2,3-triazole subunit has a negative effect on the photophysical properties of the chelate. This should be taken into consideration when planning synthesis of lanthanide(III) chelates.

Experimental Part

General. The alkyl azides **13a** – **c** were prepared from the corresponding chlorides or bromides by the method described in [3b]. Fmoc-*p*-azidophenylalanine was purchased from *Bachem*. Petroleum ether of b.p. 40 – 60° was used. Column chromatography (CC): silica gel *60* (*Merck*). UV Spectra: *Shimadzu 2400*; λ_{max} in nm, ε in $dm^3\ m^{-1}\ cm^{-1}$. Photophysical properties of the chelates prepared were measured as described previously [9a]. Luminescence measurements: *PerkinElmer-LS-55* spectrometer equipped with a *Hamamatsu-R928* red-sensitive photomultiplier tube. IR Spectra: *PerkinElmer-Spectrum-One*.

^{1}H-NMR Spectra: *Jeol-LA-600* spectrometer, at 600.0 MHz; δ(H) in ppm rel. to Me_4Si, coupling constants *J* in Hz. ESI-TOF-MS: *Applied-Biosystems-Mariner* spectrometer; in *m*/*z*.

*Tetra(*tert*-butyl) 2,2′,2″,2‴-[(4-Iodo-1*H*-pyrazole-1,3-diyl)bis(pyridine-6,2-diylmethylenenitrilo)]tetrakis[acetate]* (= N,N-*[(4-Iodo-1*H*-pyrazole-1,3-diyl)bis(pyridine-6,2-diylmethylene)]bis[*N*-[2-(1,1-dimethylethoxy)-2-oxoethyl]glycine] Bis(1,1-dimethylethyl) Ester*; **2**). To a suspension of **1** (2.0 g, 3.4 mmol) in dry MeCN (50 ml), ^{i}Pr$_2$NEt (8.9 ml, 15.3 mmol) and *tert*-butyl bromoacetate (2.3 ml, 5.1 mol) were added, and the mixture was heated overnight at 60°. The mixture was cooled to r.t. and filtered and the solid washed with MeCN. The filtrate was concetrated, the residue dissolved in CH_2Cl_2, the soln. washed with sat. $NaHCO_3$ soln., dried (Na_2SO_4), and concentrated, and the residue purified by CC (SiO_2, petroleum ether/AcOEt 3 :97): 1.1 g (38%) of **2**. ^{1}H-NMR ($CDCl_3$): 8.75 (*s*, 1 H); 7.93 (*d*, *J* = 7.6, 2 H); 7.83 (*t*, *J* = 7.4, 1 H); 7.79 (*t*, *J* = 7.6, 1 H); 7.74 (*d*, *J* = 7.6, 1 H); 7.57 (*d*, *J* = 7.7, 1 H); 4.17 (*s*, 2 H); 4.07 (*s*, 2 H); 3.59 (*s*, 4 H); 3.54 (*s*, 4 H); 1.49 (*s*, 18 H); 1.48 (*s*, 18 H). ESI-TOF-MS: 863.32 ($[M+H]^+$, $C_{39}H_{56}IN_6O_8^+$; calc. 863.32).

*Tetra(*tert*-butyl) 2,2′,2″,2‴-{{4-[(Trimethylsilyl)ethynyl]-1*H*-pyrazole-1,3-diyl}bis(pyridine-6,2-diylmethylenenitrilo)}tetrakis[acetate]* (= N,N′-*{{4-[(Trimethylsilyl)ethynyl]-1*H*-pyrazole-1,3-diyl}bis(pyridine-6,2-diylmethylene)}bis[*N*-[2-(1,1-dimethylethoxy)-2-oxoethyl]glycine] Bis(1,1-dimethylethyl) Ester*; **3**). Compound **2** (290 mg, 0.34 mmol) was co-evaporated twice from dry MeCN and then dissolved in a mixture of anh. THF (3 ml) and Et_3N (2.25 ml). Bis(triphenylphosphine)palladium(II) chloride (5 mg, 2 mol-%) and copper(I) iodide (3 mg, 4 mol-%) were added, after which the mixture was flushed with Ar for 15 min. Finally, ethynyltrimethylsilane (144 µl, 1.02 mmol) was added, and the reaction was allowed to proceed overnight at 60° in the dark. The soln. was diluted with $CHCl_3$, washed with H_2O (3×), dried (Na_2SO_4), and concentrated. The product was isolated by CC (SiO_2; petroleum ether/AcOEt 5 :4): 0.22 g (79%) of **3**. Yellowish oil. ^{1}H-NMR ($CDCl_3$): 8.83 (*s*, 1 H); 8.15 (*m*, 1 H); 8.03 (*d*, *J* = 8.2, 1 H); 7.82 (*t*, *J* = 7.6, 1 H); 7.78 (*m*, 2 H); 7.54 (*d*, *J* = 7.6, 1 H); 4.20 (*s*, 2 H); 4.05 (*s*, 2 H); 3.55 (*s*, 4 H); 3.54 (*s*, 4 H); 1.49 (*s*, 18 H); 1.47 (*s*, 18 H); 0.27 (*s*, 9 H).

*Tetra(*tert*-butyl) 2,2′,2″,2‴-[(4-Ethynyl-1*H*-pyrazole-1,3-diyl)bis(pyridine-6,2-diylmethylenenitrilo)]tetrakis[acetate]* (= N,N′-*[(4-Ethynyl-1*H*-pyrazole-1,3-diyl)bis(pyridine-6,2-diylmethylene)]bis[*N*-[2-(1,1-dimethylethoxy)-2-oxoethyl]glycine] Bis(1,1-dimethylethyl) Ester*; **4**). Compound **3** (220 mg, 0.26 mmol), dissolved in CH_2Cl_2 (2 ml) was treated with Bu_4NF (138 mg, 0.53 mmol). After 2 h stirring at r.t., the solvent was evaporated. Purification by CC (SiO_2, petroleum ether/AcOEt 5 :4) gave 0.13 g (60%) of **4**. Colorless oil. ^{1}H-NMR ($CDCl_3$): 8.84 (*s*, 1 H); 8.06 (*d*, *J* = 7.4, 1 H); 8.00 (*d*, *J* = 8.0, 1 H); 7.82 (*t*, *J* = 7.9, 1 H); 7.78 (*t*, *J* = 7.7, 1 H); 7.71 (*d*, *J* = 7.7, 1 H); 7.55 (*d*, *J* = 7.3, 1 H); 4.16 (*s*, 2 H); 4.05 (*s*, 2 H); 3.56 (*s*, 4 H); 3.53 (*s*, 4 H); 3.25 (*s*, 1 H); 1.48 (*s*, 18 H); 1.46 (*s*, 18 H). ESI-TOF-MS: 761.41 ($[M+H]^+$, $C_{41}H_{57}N_6O_8^+$; calc. 761.42).

*Tetra(*tert*-butyl) 2,2′,2″,2‴-{{4′-{5-[(Trimethysilyl)ethynyl]furan-2-yl}[2,2′:6′,2″-terpyridine]-6,6″-diyl}bis(methylenenitrilo)}tetrakis[acetate]* (= N,N′-*{{4′-{5-[(Trimethylsilyl)ethynyl]furan-2-yl}[2,2′:6′,2″-terpyridine]-6,6″-diyl}bis(methylene)}bis[*N*-[2-(1,1-dimethylethoxy)-2-oxoethyl]glycine] Bis(1,1-dimethylethyl) Ester*; **8**). As described above for **3**, with **7**. Purification was performed by CC (SiO_2, petroleum ether/AcOEt/Et_3N 5 :3 :1): **8** (77%). ^{1}H-NMR ($CDCl_3$): 8.70 (*s*, 2 H); 8.50 (*d*, *J* = 7.3, 2 H); 7.85 (*t*, *J* = 7.6, 2 H); 7.72 (*d*, *J* = 7.3, 2 H); 7.09 (*d*, *J* = 3.5, 1 H); 6.77 (*d*, *J* = 3.5, 1 H); 4.18 (*s*, 4 H); 3.55 (*s*, 8 H); 1.48 (*s*, 36 H); 0.31 (*s*, 9 H).

*Tetra(*tert*-butyl) 2,2′,2″,2‴-{[4′-(5-Ethynylfuran-2-yl)[2,2′:6′,2″-terpyridine]-6,6″-diyl]bis(methylenenitrilo)}tetrakis[acetate]* (= N,N′-*{[4′-(5-Ethynylfuran-2-yl)[2,2′:6′,2″-terpyridine]-6,6″-diyl]bis(methylene)}bis[*N*-[2-(1,1-dimethylethoxy)-2-oxoethyl]glycine] Bis(1,1-dimethylethyl) Ester*; **9**). As described above for **4**. Purification was performed by CC (SiO_2, petroleum ether/AcOEt/Et_3N 5 :4 :1): **9** (68%). ^{1}H-NMR ($CDCl_3$): 8.71 (*s*, 2 H); 8.51 (*d*, *J* = 7.7, 2 H); 7.86 (*t*, *J* = 7.9, 2 H); 7.72 (*d*, *J* = 7.7, 2 H); 7.11 (*d*, *J* = 3.5, 1 H); 6.82 (*d*, *J* = 3.5, 1 H); 4.19 (*s*, 4 H); 3.56 (*s*, 8 H); 3.53 (*s*, 1 H); 1.48 (*s*, 36 H). ESI-TOF-MS: 838.45 ($[M+H]^+$, $C_{41}H_{57}N_6O_8^+$; calc. 838.44).

Cycloaddition Reaction: General Procedure. The alkyne (0.8 mmol) and azide (2 mol-equiv.) were dissolved in DMSO (3 ml). The mixture was flushed with Ar, and copper(I) iodide (3 mg, 10 mol-%) was added. The reaction was conducted overnight at 60° in the dark. DMSO was evaporated (oil pump). The residue was dissolved in CH_2Cl_2, the soln. washed with 10% H_4edta, dried (Na_2SO_4), and evaporated, and the residue purified by CC: **5**, **10**, or **14a–c**.

*Tetra(*tert*-butyl) 2,2′,2″,2‴-{{4-[1-(3-Hydroxypropyl)-1*H*-1,2,3-triazol-4-yl]pyrazole-1,3-diyl}bis(pyridine-6,2-diylmethylenenitrilo)}tetrakis[acetate]* (=N,N′*-{{4-[1-(3-Hydroxypropyl)-1*H*-1,2,3-triazol-4-yl]pyrazole-1,3-diyl}bis(pyridine-6,2-diylmethylene)}bis[*N*-[2-(1,1-dimethylethoxy)-2-oxoethyl]glycine] Bis(1,1-dimethylethyl) Ester*; **5**). CC (SiO_2, 2→5% MeOH/CH_2Cl_2) gave **5** (59%). ^{1}H-NMR ($CDCl_3$): 9.32 (*s*, 1 H); 8.94 (*s*, 1 H); 8.08 (*d*, *J* = 8.0, 1 H); 7.96 (*d*, *J* = 7.9, 1 H); 7.83 (*t*, *J* = 7.4, 1 H); 7.78 (*t*, *J* = 7.7, 1 H); 7.61 (*d*, *J* = 7.3, 1 H); 7.53 (*d*, *J* = 7.6, 1 H); 4.63 (*t*, *J* = 6.5, 2 H); 4.22 (*s*, 2 H); 4.06 (*s*, 2 H); 3.70 (*t*, *J* = 5.3, 2 H); 3.53 (*s*, 4 H); 3.49 (*s*, 4 H); 2.22 (*m*, 2 H); 1.48 (*s*, 18 H); 1.46 (*s*, 18 H). ESI-TOF-MS: 862.45 ($[M+H]^+$, $C_{44}H_{63}N_9O_9^+$; calc. 862.47).

*Tetra(*tert*-butyl) 2,2′,2″,2‴-{{4′-{5-[1-(3-Hydroxypropyl)-1*H*-1,2,3-triazol-4-yl]furan-2-yl}[2,2′:6′,2″-terpyridine]-6,6″-diyl}bis(methylenenitrilo)}tetrakis[acetate]* (=N,N′*-{{4′-{5-[1-(3-Hydroxypropyl)-1*H*-1,2,3-triazol-4-yl]furan-2-yl}[2,2′:6′,2″-terpyridine]-6,6″-diyl}bis(methylene)}bis[*N*-[2-(1,1-dimethylethoxy)-2-oxoethyl]glycine] Bis(1,1-dimethylethyl) Ester*; **10**). CC (SiO_2, 2→5% MeOH/CH_2Cl_2) gave **10** (58%). ^{1}H-NMR ($CDCl_3$): 8.75 (*s*, 2 H); 8.53 (*d*, *J* = 7.9, 2 H); 8.20 (*s*, 1 H); 7.86 (*t*, *J* = 7.6, 2 H); 7.67 (*d*, *J* = 7.6, 2 H); 7.22 (*d*, *J* = 3.5, 1 H); 7.07 (*d*, *J* = 3.5, 1 H); 4.68 (*t*, *J* = 6.5, 2 H); 4.21 (*s*, 4 H); 3.73 (*t*, *J* = 5.9, 2 H); 3.59 (*s*, 8 H); 2.21 (*m*, 2 H); 1.46 (*s*, 36 H). ESI-TOF-MS: 939.49 ($[M+H]^+$, $C_{50}H_{67}N_8O_{10}^+$; calc. 939.50).

*Tetra(*tert*-butyl) 2,2′,2″,2‴-{{4-[1-(3-Hydroxypropyl)-1*H*-1,2,3-triazol-4-yl]pyridine-2,6-diyl}bis(methylenenitrilo)}tetrakis[acetate]* (=N,N′*-{{4-[1-(3-Hydroxypropyl)-1*H*-1,2,3-triazol-4-yl]pyridine-2,6-diyl}bis(methylene)}bis[*N*-[2-(1,1-dimethylethoxy)-2-oxoethyl]glycine] Bis(1,1-dimethylethyl) Ester*; **14a**). CC (SiO_2, petroleum ether/AcOEt/Et_3N 2:5:1) gave **14a** (79%). ^{1}H-NMR ($CDCl_3$): 8.08 (*s*, 1 H); 7.95 (*s*, 2 H); 4.59 (*t*, *J* = 6.8, 2 H); 4.06 (*s*, 4 H); 3.67 (*t*, *J* = 5.9, 2 H); 3.51 (*s*, 8 H); 2.18 (*t*, *J* = 6.0, 2 H); 1.45 (*s*, 36 H). ESI-TOF-MS: 719.43 ($[M+H]^+$, $C_{36}H_{59}N_6O_9^+$; calc. 719.43).

*Tetra(*tert*-butyl) 2,2′,2″,2‴-{{4-[1-(2-Carboxyethyl)-1*H*-1,2,3-triazol-4-yl]pyridine-2,6-diyl}bis(methylenenitrilo)}tetrakis[acetate]* (=N,N′*-{{4-[1-(2-Carboxyethyl)-1*H*-1,2,3-triazol-4-yl]pyridine-2,6-diyl}bis(methylene)}bis[*N*-[2-(1,1-dimethylethoxy)-2-oxoethyl]glycine] Bis(1,1-dimethylethyl) Ester*; **14b**). CC (SiO_2, MeOH/CH_2Cl_2 15:85) gave **14b** (35%). ^{1}H-NMR ((D$_6$)DMSO): 8.51 (*s*, 1 H); 7.60 (*s*, 2 H); 4.76 (*m*, 2 H); 3.88 (*s*, 4 H); 3.39 (*s*, 8 H); 2.82 (*m*, 2 H); 1.44 (*s*, 36 H). ESI-TOF-MS: 733.41 ($[M+H]^+$, $C_{35}H_{55}N_6O_{10}^+$; calc. 733.41).

*Tetra(*tert*-butyl) 2,2′,2″,2‴-{{4-{1-{4-{2-Carboxy-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}ethyl}phenyl}-1*H*-1,2,3-triazol-4-yl}pyridine-2,6-diyl}bis(methylenenitrilo)}tetrakis[acetate]* (=N,N′*-{{4-{1-{4-{2-Carboxy-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}ethyl}phenyl}-1*H*-1,2,3-triazol-4-yl}pyridine-2,6-diyl}bis(methylene)}bis[*N*-[2-(1,1-dimethylethoxy)-2-oxoethyl]glycine] Bis(1,1-dimethylethyl) Ester*; **14c**). CC (SiO_2, AcOH/MeOH/CH_2Cl_2 0.25:15:85) gave **14c** (30%). ^{1}H-NMR ((D$_6$)DMSO): 9.33 (*s*, 1 H); 7.93 (*s*, 1 H); 7.87 (*s*, 2 H); 7.84 (*d*, *J* = 7.7, 2 H); 7.78 (*d*, *J* = 7.9, 2 H); 7.60 (*t*, *J* = 7.7, 2 H); 7.36 (*m*, 4 H); 7.27 (*m*, 2 H); 4.29 (*m*, 1 H); 4.15 (*m*, 2 H); 3.95 (*s*, 4 H); 3.45 (*s*, 8 H); 1.36 (s, 36 H). ESI-TOF-MS: 1046.51 ($[M+H]^+$, $C_{57}H_{72}N_7O_{12}^+$; calc. 1046.52).

Lanthanide(III) Chelates **6a**–**c** and **11**. The esters **5** and **10** were converted to the corresponding lanthanide(III) chelates by the method described in [13].

*{2,2′,2″,2‴-{{4-[1-(3-Hydroxypropyl)-1*H*-1,2,3-triazol-4-yl]pyrazole-1,3-diyl}bis(pyridine-6,2-diylmethylenenitrilo)}tetrakis[acetato]}europium(III)* (=*{*N,N′*-{{4-[1-(3-Hydroxypropyl)-1*H*-1,2,3-triazol-4-yl]pyrazole-1,3-diyl}bis(pyridine-6,2-diylmethylene)}bis[*N*-(carboxymethyl)glycinato](4−)}europate(1−)*; **6a**). UV (H_2O): 321 (14400). ESI-TOF-MS: 889.18 ($[M+Et_3N]^+$, $C_{34}H_{44}EuN_{10}O_9^+$; calc. 889.25).

*{2,2′,2″,2‴-{{4-[1-(3-Hydroxypropyl)-1*H*-1,2,3-triazol-4-yl]pyrazole-1,3-diyl}bis(pyridine-6,2-diylmethylenenitrilo)}tetrakis[acetato]}terbium(III)* (=*{*N,N′*-{{4-[1-(3-Hydroxypropyl)-1*H*-1,2,3-triazol-4-yl]pyrazole-1,3-diyl}bis(pyridine-6,2-diylmethylene)}bis[*N*-(carboxymethyl)glycinato](4−)}terbate(1−)*; **6b**). UV (H_2O): 323 (14400). ESI-TOF-MS: 895.27 ($[M+Et_3N]^+$, $C_{34}H_{44}N_{10}O_9Tb^+$; calc. 895.25).

*{2,2′,2″,2‴-{{4-[1-(3-Hydroxypropyl)-1*H*-1,2,3-triazol-4-yl]pyrazole-1,3-diyl}bis(pyridine-6,2-diylmethylenenitrilo)}tetrakis[acetato]}dysprosium(III)* (=*{*N,N′*-{{4-[1-(3-Hydroxypropyl)-1*H*-1,2,3-triazol-4-yl]pyrazole-1,3-diyl}bis(pyridine-6,2-diylmethylene)}bis[*N*-(carboxymethyl)glycinato](4−)}disprosate(1−)*; **6c**). UV (H_2O): 323 (11500). ESI-TOF-MS: 899.31 ($[M+Et_3N]^+$, $C_{34}H_{44}DyN_{10}O_9^+$; calc. 899.25).

*{2,2′,2″,2‴-{{4′-{5-[1-(3-Hydroxypropyl)-1*H*-1,2,3-triazol-4-yl]furan-2-yl}[2,2′:6′,2″-terpyridine]-6,6″-diyl}bis(methylenenitrilo)}tetrakis[acetato]}europium(III)* (=*{*N,N′*-{{4′-{5-[1-(3-Hydroxypropyl)-*

*1*H-*1,2,3-triazol-4-yl]furan-2-yl}[2,2′:6′,2″-terpyridine]-6,6″-diyl}bis(methylene)}bis[*N-*(carboxymethyl)glycinato](4−)}europate(1−)*; (**11**). UV (H_2O): 357 (14700). ESI-TOF-MS: 863.07 ($[M]^-$, $C_{34}H_{30}EuN_8O_{10}^-$; calc. 863.13).

*{2,2′,2″,2‴-{[4-(3-Hydroxypropyl)-1*H*-pyrazole-1,3-diyl]bis(pyridine-6,2-diylmethylenenitrilo)}tetrakis[acetato]}terbium(III)* (=*{*N,N′*-{[4-(3-Hydroxypropyl)-1*H*-pyrazole-1,3-diyl]bis(pyridine-6,2-diylmethylene)}bis[*N*-(carboxymethyl)glycinato](4−)}terbate(1−)*; **15**). Tetramethyl 2,2′,2″,2‴-{[4-(3-hydroxypropyl)-1*H*-pyrazole-1,3-diyl]bis(pyridine-6,2-diylmethylenenitrilo)}tetrakis[acetate] [11] was dissolved in 0.2M KOH/MeOH, a drop of H_2O was added, and the mixture was stirred for 4 h at r.t. The pH was adjusted to 6.5 with 0.1M HCl, and the resulting ligand was converted to the terbium(III) chelate as described in [13]. UV (H_2O): 327 (18700). ESI-TOF-MS: 725.01 (M^-, $C_{26}H_{26}N_6O_9Tb^-$; calc. 725.10).

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Received December 30, 2006