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6,6′-Dimethyl-2,2′-bipyridine-4-ester: A pivotal synthon for building tethered bipyridine ligands

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ABSTRACT

We describe an efficient and scalable synthesis of 4-carbomethoxy-6,6′-dimethyl-2,2′-bipyridine starting from easily available substituted 2-halopyridines and based on the application of modified Negishi cross-coupling conditions. This compound is a versatile starting material for the synthesis of 4-functionalized 2,2′-bipyridines bearing halide, alcohol, amine, and other functionalities, suitable for conjugation to biological material (**2a**–**c**, **3a**–**g**). The utility of this compound in the construction of more complex architectures was further demonstrated by the synthesis of two bifunctional lanthanide chelators; an open chain ligand based on one 2,2′-bipyridine unit and a cryptand based on three 2,2′-bipyridine units [N_2(bpy)$_3$COOMe]. In the field of luminophoric biolabels, the photophysical properties of the corresponding Eu(III) cryptate are reported.

1. Introduction

Much attention is given to 2,2′-bipyridine (bpy) systems because of their central role in the fields of inorganic, organometallic, and coordinations chemistries. Bpy ligands generally afford efficient binding affinity for most elements in the periodic table due to the chelation effect and the π-accepting ability of these moieties.[1] It was shown that the transition metal–bpy complexes found various applications in many areas of chemistry including catalysis,[2] electrochemistry,[3] artificial photosynthesis,[4] luminescent sensor molecules,[5] nonlinear optical (NLO) materials[6] and organic light emitting diodes (OLEDs).[7] Besides, bpy derivatives are attractive building blocks for supramolecular chemistry and macromolecular chemistry.[8] Lanthanide complexes of bpy-based ligands have been also the focus of much attention owing to their important applications as time-resolved luminescence labels in bioanalyses and medical diagnostic.[9–11] As a matter of fact, bpy fragments act as effective sensitizer for causing visible luminescence from Eu(III), Tb(III), Sm(III), Dy(III) centers and near-infrared (NIR) luminescence from Yb(III) and Nd(III) centers.[12–15] In particular, the luminescence properties of the europium complex of the Lehn cryptand [N_2(bpy)$_3$] are well established[16] while bioassays using a such probe have considerable commercial application. It has been applied as a luminescent label in direct homogeneous immunoassays, in in situ hybridization and in PCR detection, and finds wide applications in diagnostic and high throughput screening for drug discovery (HTRF® technology).[17] Functionalized bpy derivatives have commonly been required for all the purposes described above. Especially, the covalent attachment of lanthanide organocomplexes to biological materials is an important step in the development of luminescent bioprobes. In this direction, 2,2′-bipyridine is an ideal core for introduction of a single conjugation group opposite to the complexing sites of the molecule. The introduction of a single reactive group precludes undesirable intra- and intermolecular cross-linkings in conjugation reactions with biomolecules. [18–20] As a matter of fact, cross-linking may have dramatic impact on the biological and immunogenic properties of the ligand-biomolecule conjugate. The already described procedures of Mukkala et al. afford access to 4-substituted 2,2′-bipyridine derivatives that allow bioconjugation of luminescent lanthanide chelates.[21] In this work, the introduction of a single active functionality at the 4-position was performed via a multistep procedure including the mono-*N*-oxide formation of 6,6′-dimethyl-2,2′-bipyridine, the subsequent introduction of a nitro group at the 4-position of bpy and further derivatizations of the nitro functionality to create derivatives suitable for bioconjugation. Nevertheless, the synthesis of 4-

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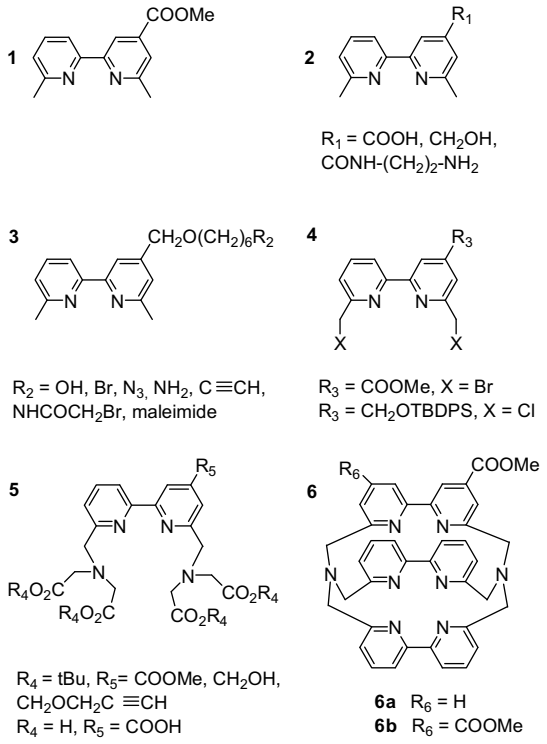
substituted-bpy moieties has been much less explored that ones that are symmetrically disubstituted at the 4,4′-position.[22] As a consequence, limited accessibility to unsymmetrically functionalized bpy derivatives has restricted their potential use in the construction of more sophisticated architectures.

As part of a research program aimed at the design and synthesis of lanthanide luminescence bioprobes,[12,13,23] we were interested in the synthesis of ligands based on a bpy moiety containing an appropriate monofunctionalization to be covalently attached to bioactive molecules. In this direction, 4-carbomethoxy-6,6′-dimethyl-2,2′-bipyridine (**1**, Scheme 1) is an useful synthon.



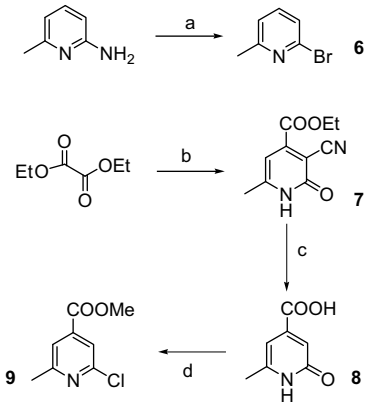
Scheme 1. Structures of 2,2′-bipyridine derivatives.

In this compound, the aromatic ester group opens avenues to the introduction of various functional groups suitable for biological labeling. The methyl groups are available for different functionalization reactions allowing the introduction of bpy unit in multifunctional chelating agents. Despite their prevalence, the synthesis of 6,6′-dimethyl-2,2′-bipyridine compound bearing an ester function at the 4-position was reported only recently. The methyl ester derivative **1** was prepared from a Stille-type cross-coupling reaction requiring a pyridyltriflate derivative as coupling component to provide satisfying yield.[24] The corresponding ethyl ester derivative was prepared by using a combination of Stille and Negishi cross-coupling methodologies with a moderate yield.[25] In this report we describe a full account of our efforts to develop a scalable and friendly preparation of **1** and the achievement of this goal via a Negishi's zinc coupling.[26] We have also investigated the utility of the methyl ester function and methyl groups of **1** by elaboration of a number of 6,6′-dimethyl-2,2′-bipyridine derivatives asymmetrically substituted at the 4-position with various moieties (compounds **2** and **3**, Scheme 1) and by the conversion of the methyl groups into bromomethyl and chloromethyl functions (compounds **4**, Scheme 1). As examples of practical applications in the direction of time-resolved luminescent Ln(III) probes, we describe the further elaboration of the monofunctionalized open chain ligands (**5**) and the $[N_2(bpy)_3(CO_2Me)]$ cryptand (**6a**). The photophysical properties of **6a**·Eu^{3+} cryptate are presented and compared to those of the corresponding bifunctionalized $[N_2(bpy)_3(CO_2Me)_2$·Eu] cryptate (**6b**·Eu^{3+}), commercially available for HTRF® assays.

2. Results and discussion

2.1. Synthesis of 4-carbomethoxy-6,6′-dimethyl-2,2′-bipyridine (1)

In principle, the synthesis of the targeted bpy compound (**1**), 4-carbomethoxy-6,6′-dimethyl-2,2′-bipyridine, can mainly be achieved through three different routes: a) introduction of the functional ester group into symmetrical bpy derivative (6,6′-dimethyl-2,2′-bipyridine), b) ring-assembly methodology like the Kröhnke procedure or its various procedures, c) preliminary functionalization of two different pyridine components followed by coupling methodology generally employed for the formation of aryl–aryl bonds. The two former approaches are limited by not easily accessible starting compounds, multi-step procedures with low yields and (or) require harsch conditions.[27,28] For the latter approach, palladium-catalyzed cross-coupling procedures, such as Stille-type and Negishi-type allow a more flexible preparation of unsymmetrically substituted and functionalized bipyridines.[29–32] On this basis, the synthesis of **1** was approached via a metal-catalyzed coupling of the two pyridine fragments **6** and **9**, which are amenable to large laboratory scale preparations (Scheme 2). The synthetic strategy for preparing these two starting materials is depicted in Scheme 2.

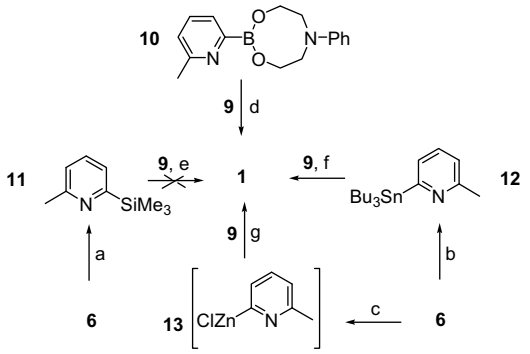


Scheme 2. Preparation of starting materials. Reagents and conditions; (a) HBr, Br_2, $NaNO_2$, H_2O, −20 °C (75%); (b) i) EtONa, rt, 12 h; ii) cyanoacetamide, 80 °C, 6 h; iii) acetic acid (68%); (c) HCl 6 N, reflux, 24 h (91%); (d) i) $POCl_3$, reflux, 18 h; ii) MeOH, rt, 24 h (83%).

The reagent 2-bromo-6-methylpyridine **6** can be obtained commercially or synthesized from cheap 2-amino-5-methylpyridine by a Sandmeyer/halogenation sequence with HBr, bromine and sodium nitrite.[33] This reaction could be adapted to a 150 g scale using a literature procedure.[34] The trifunctionalized pyridine **9**, bearing an ester group was obtained in three steps starting from diethyl oxalate by improvement of literature procedures.[35–37] In a first step, the sodium salt of ethyl acetopyruvate was generated in situ by the treatment of diethyl oxalate with acetone in the presence of sodium ethoxide and this was reacted with cyanoacetamide to give the 2-pyridone derivative **7** in 68% yield. Upon hydrolysis of the nitrile group and concomitant loss of carbon dioxide of **7** in aqueous 6 N HCl, acide **8** was produced in 91% yield. Using this route, **8** can be readily prepared in a multi-gram scale using a simple precipitation procedure at each step for purification. Treatment of **8** with phosphorous oxychloride following by methanol then afforded the targeted pyridyl derivative **9** in 83% yield after a short column chromatography on silica gel. Compound **9**

was thus obtained with an overall yield of 51% starting from diethyl oxalate.

With compounds **6** and **9** in hands, we began our synthetic studies by finding the optimal methodology for the desired cross-coupling of these two partners (Scheme 3).

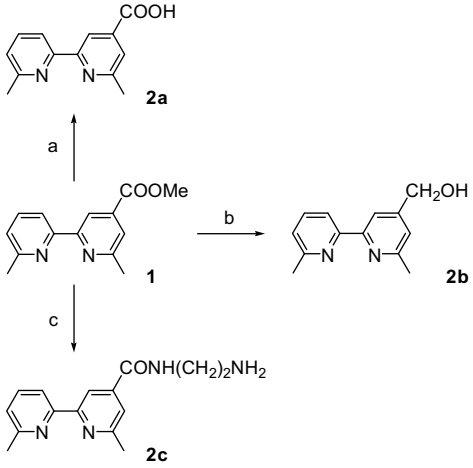


Scheme 3. Syntheses of **1** used in this study. Reagents and conditions: (a) i) *n*BuLi, THF, −78 °C; ii) Me_3SiCl, rt, 18 h (55%); (b) i) *n*BuLi, THF, −78 °C; ii) Bu_3SnCl, rt, overnight (96%); (c) i) *n*BuLi, THF, −78 °C; ii) $ZnCl_2$, rt, 0.5 h; (d) $PdCl_2(PPh_3)_2$, K_3PO_4, CuI, DMF, 100 °C, 8 h (45%); (e) $PdCl_2(PPh_3)_2$, PPh_3, CuI, DMF, 120 °C, 48 h according to Ref 40; (f) $Pd(PPh_3)_4$, toluene, reflux, 48 h (30–66%); (g) $PdCl_2(PPh_3)_2$, DIBALH, THF, reflux, 4 h (83%).

Firstly, in order to carry out cross-coupling reactions under Suzuki–Miyaura conditions, we used boronic ester **10** which has been shown to be stable and is now commercially available.[38] According to the procedure of Jones et al.,[39] the coupling of 1.2 equiv of boronic ester **10** relative to chloropyridine **9** was performed in the presence of 5 mol % $PdCl_2(PPh_3)_2$ and CuI. In these conditions, the desired bpy product **1** was obtained in modest isolated yield (45%). The next attempts were made using the Hiyama cross-coupling, between **9** and pyridylsilane **11**, prepared from **6** via halogen–lithium exchange and subsequent reaction with chlorotrimethylsilane. By using the catalyst system recently proposed by Pierrat et al. ($PdCl_2(PPh_3)_2$/PPh_3/CuI) for the palladium-catalysed Hiyama cross-coupling of 2-bromopyridine and chloropyridyltrimethyl silanes,[40] these attempts were unsuccessful and no cross-coupling product was isolated. These results highlighted again that the lack of electron-withdrawing substituent on the pyridine ring of 2-trimethylsilylpyridines prevents the formation of the final aryl–aryl bond.[40] We have also used the 2-tributylstannylpicoline **12**, prepared as previously reported,[41] as reactive partner in a Stille-type reaction. Cross-coupling reaction was carried out in degassed toluene in the presence of a catalytic amount of $Pd(PPh_3)_4$ (5 mol %), and afforded the desired bipyridine **1**, but yields tend to be variable (30–60%). Although a classical acidic aqueous workup was employed for removing tin byproducts,[42] the use of stannane compound give rises to difficulties in product purification (this may in part be responsible for the variation in yield). The preparation of **1** by using Negishi-type chemistry and organozinc reagent was more convenient, being more reliable and providing a better yield. The pyridylzinc chloride compound **13** was satisfactorily prepared from bromide **6** by halogen–metal exchange with *n*-butyllithium, followed by transmetallation with $ZnCl_2$. Heterocoupling reaction of **13** with **9** in the presence of 5 mol % of a catalyst[43] prepared from $PdCl_2(PPh_3)_2$ and DIBALH (2 equiv) in refluxing THF afforded compound **1** in 83% yield after column chromatography. This process also benefits from the fact that it is conveniently carried out in a single pot and allows the extension to large-scale preparations (up to 10 g) of the versatile bipyridine **1**, with no significant loss of yield or efficiency. Finally, it is worth mentioning that we also tried to prepare **1** by using a simple iron salt as environmentally friendly catalyst. A precedent[44] from Fuernstner's group for the $Fe(acac)_3$ catalyzed coupling of chloroquinoxaline and 3-pyridylmagnesium bromide inspired us to attempt a similar coupling through Grignard reagent derived from **6**. Disappointingly, no coupling product was isolated.

2.2. Compound 1: derivatization of the aromatic ester group

The pivotal building block **1** with an ester group can be used as a synthetic handle to introduce other functional groups such as carboxylic acid, alcohol, amine (Scheme 4).



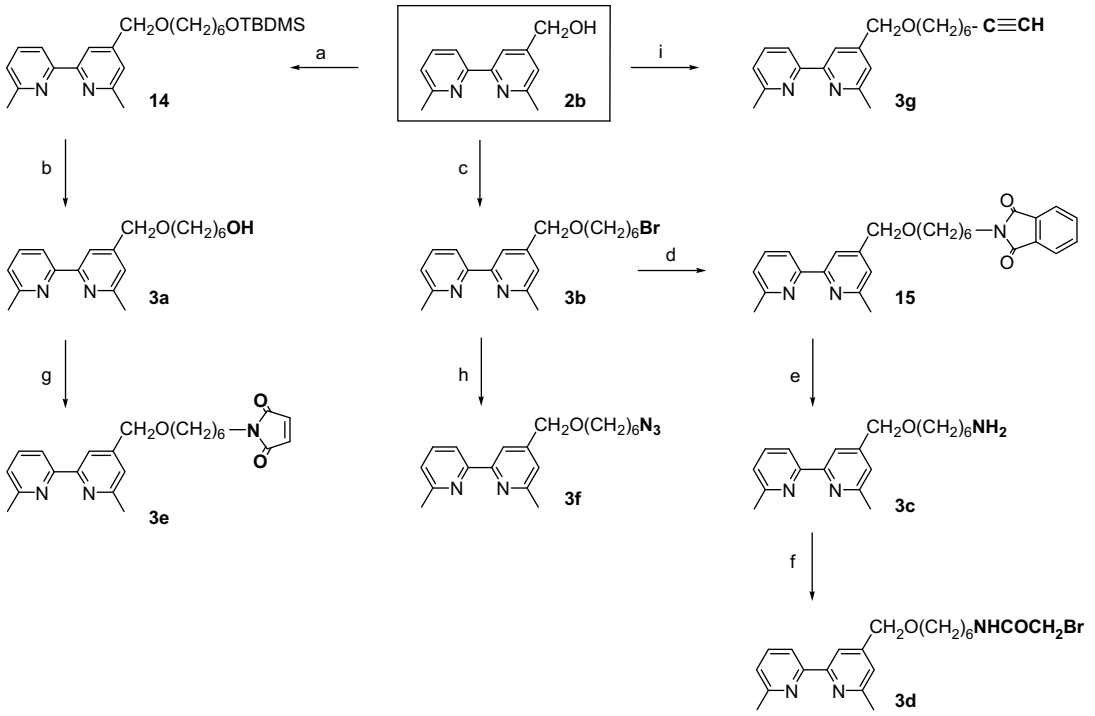
Scheme 4. Derivatization of **1**. Reagents and conditions. (a) K_2CO_3, MeOH/H_2O, 80 °C, 0.5 h (100%); (b) $NaBH_4$, EtOH, rt, 16 h (82%); (c) $NH_2CH_2CH_2NH_2$, rt, 24 h (100%).

The ester function was hydrolysed in mild basic conditions to the corresponding acid **2a** and was easily reduced to hydroxymethyl group (**2b**) with $NaBH_4$ in methanol. Moreover, the reaction of the ester compound **1** with a large excess (30 equiv) of neat ethylenediamine produces the aminoethylamide **2c** cleanly and quantitatively after 24 h at room temperature. It is worth noting that the amido-amino compound **2c** could be further coupled for vectorisation to various biomolecules using commercial heterobifunctional cross-linking reagents.[17,45]

The hydroxymethyl function of **2b** offers another potential source of further derivatization, through a biologically stable ether linkage. In this direction, a representative series of bpy derivatives bearing alcohol, bromide, amine and other functionalities, and a classical alkyl tether of six atoms spacer was obtained (Scheme 5).

Access to the hydroxyl-terminated bpy derivative **3a** required deprotonation of **2b** with NaH and reaction of the resultant alcoholate with commercial (6-bromohexyloxy)-*tert*-butyldimethylsilane. The resulting ether **14** was cleanly deprotected with TBAF to yield target alcohol in 46% yield over the two steps from **2b**. Similarly, after deprotonation of **2b**, the direct preparation of bromo-terminated derivative **3b** was possible utilizing a 2.6-fold excess of 1,6-dibromohexane. In this way compound **3b** can be prepared in 50% yield. According to the classical Gabriel synthesis methodology, amino-terminated bpy derivative **3c** was prepared from **3b** in a two step procedure via its phthalimido precursor **15**. The purified yield of **15** was 78%, and this was converted into **3c** in 96% yield by treatment with hydrazine hydrate.

Derivatizing bpy agents containing haloacetamidyl (**3d**) or maleimide (**3e**) moiety were also prepared. These electrophilic groups are commonly used in biological setting and react selectively with thiol groups either extant or introduced into biomolecules.[46] The bromoacetamide derivative was made from the amine **3c** using the bifunctional bromoacetyl chloride reagent (1.2 equiv) in the presence of *N*-methyl morpholine (2.5 equiv) in 72% yield. Two different routes for the preparation of the



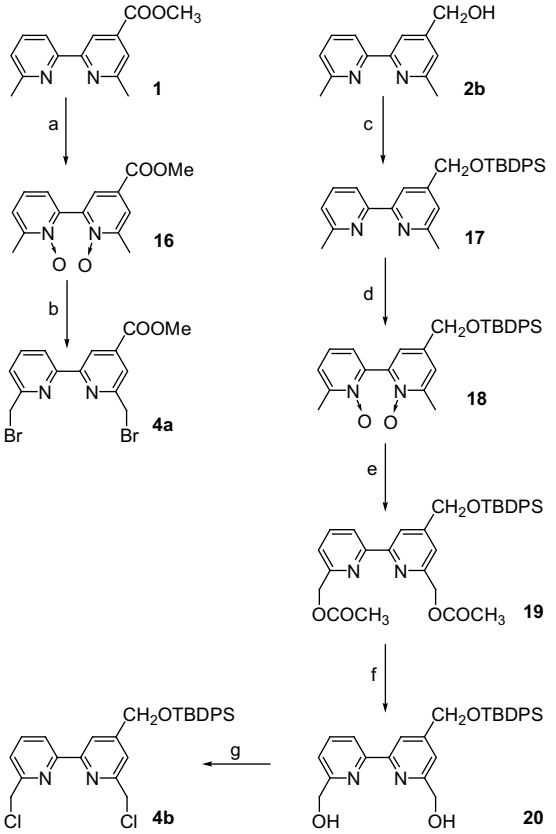
Scheme 5. Derivatization of **2b**. Reagents and conditions. (a) i) NaH, DMF, 60 °C, 1 h; ii) $Br(CH_2)_6OTBDMS$, 60 °C, 24 h (57%); (b) TBAF, THF, rt, 3 h (80%); (c) i) NaH, DMF, 60 °C, 1 h; ii) $Br(CH_2)_6Br$ (2.6 equiv), 60 °C, 20 h (50%); (d) potassium phthalimide, DMF, 100 °C, 5 h (78%); (e) NH_2NH_2, MeOH, rt, 3 h (96%); (f) $ClCOCH_2Br$, *N*-methyl morpholine, CH_2Cl_2, rt, 18 h (72%); (g) maleimide, PPh_3, DEAD, THF, 0 °C, 12 h (54%); (h) NaN_3, DMF, rt, overnight (99%); (i) i)NaH, THF, 60 °C, 1 h; ii) 8-bromo-1-octyne, reflux, 16 h (36%).

maleimide derivative **3e** were investigated. The classical method requires the reaction of amines with maleic anhydride, followed by dehydration/cyclisation of the maleimic acid intermediates, usually promoted by acid.[47] An alternative method described by Walker using alcohols as starting materials involves the direct N-alkylation of maleimide under mild Mitsunobu conditions.[48] Attempts to prepare **3e** by treating amine **3c** with maleic anhydride followed by sodium acetate in acetic acid were disappointing in terms of both yield (<20%) and reproducibility. The desired maleimide derivative can be conveniently prepared by exploiting the Mitsunobu reaction. Thus, treatment of alcohol **3a** with maleimide in the presence of DEAD and PPh_3 furnished **3e** in 54% yield.

To provide additional bpy reagents of general utility, we have also investigated the introduction of azide and alkyne moieties. These functional groups are inertness towards physiological conditions, which allows the use of the Huisgen 1,3-dipolar cycloaddition under copper (I) catalysis for in vitro and in vivo bioconjugation.[49] The bromo derivative **3b** was easily transformed into the respective azido derivative **3f** in a nearly quantitative yield by treatment with sodium azide at room temperature. The synthesis of acetylene compound **3g** began with the preparation of 8-bromo-1-octyne in a two steps procedure. Firstly, 2-octyn-1-ol was subjected to a Zipper reaction (triple bond isomerization) with mixed alkali metal amide[50] to afford terminal isomer 7-octyn-1-ol. In this latter compound, the hydroxyl group was then replaced by bromine group by reaction with NBS/PPh_3 using standard methodology, yielding 8-bromo-1-octyne in 60% yield. Reaction of this bromo acetylene compound with sodium alcoholate of **2b** afforded **3g** in moderate yield (36%).

2.3. Compounds 1 and 2b: activation of the methyl groups at the 6,6′-positions

In pivotal compounds **1** and **2b**, the methyl groups at the 6,6′-positions are available for different functionalization reactions which are depicted in Scheme 6.



Scheme 6. Activation of methyl groups of **1** and **2b**. Reagents and conditions: (a) *m*-CPBA, $CHCl_3$, rt, 19 h (95%); (b) i) Ac_2O, 120 °C, 16 h; ii) HBr, AcOH, 70 °C, 7 h (63%); (c) TBDPSCl, imidazole, DMF, rt, 48 h (94%); (d) *m*-CPBA, $CHCl_3$, rt, 24 h (78%); (e) Ac_2O, 100 °C, 24 h (68%); (f) K_2CO_3, $MeOH/H_2O$, rt, 3 h (100%); (g) $SOCl_2$, rt, 1.5 h (64%).

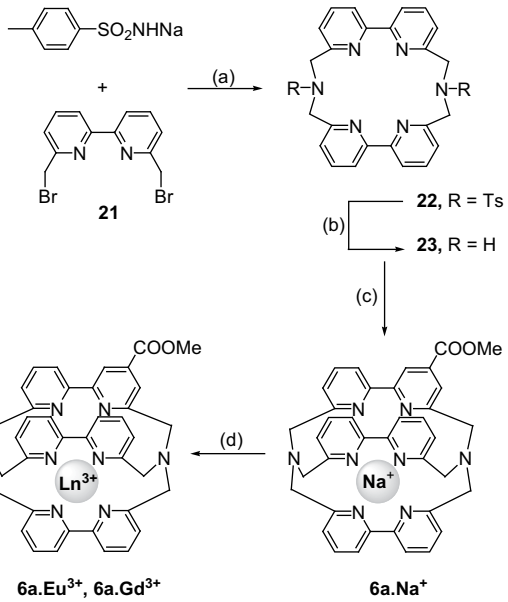
Initial attempts to obtain dibromide **4a** directly from **1** by a free-radical bromation afforded product in low yield. Specifically, **1** was refluxed in benzene under irradiation in the presence of *N*-bromosuccinimide and a catalytic amount of AIBN. All trials produced a mixture of brominated products from which the targeted compound was isolated in only 20% yield, at best, after careful purifications by column chromatography and recrystallisation. Moreover, carrying out a selective debromination of polybrominated by-products with diisobutylaluminium hydride[51] or diethylphosphite and *N*,*N*′-diisopropylethylamine,[52] only slight improvements in overall yield were obtained. Therefore, a more circuitous procedure was employed by using the well-known Boekelheide rearrangement followed by a pseudohalogen exchange.[27] The 1,1′-dioxide compound **16** was readily prepared (95%) by oxidation of **1** with 3-chloroperoxybenzoic acid (*m*-CPBA) in chloroform at room temperature. After treatment of **16** with acetic anhydride at reflux, the resulting rearranged diacetate was displaced with hydrobromic acid, 33% in acetic acid, to give dibromide **4a** in 63% yield. Stepwise conversion of **1** to **4a** was thus made in two steps with a total yield of 60%.

The monoprotected triol **20** was obtained in four steps starting from alcohol **2b** and following the methodology developed for the **1**→**4a** conversion. After protection of the hydroxyl group of **2b** with *tert*-butyldiphenylsilyl chloride in the presence of imidazole, subsequent treatment with *m*-CPBA resulted in the formation of bis *N*-oxide **18** (94 and 78% yield respectively for these two steps). When bis *N*-oxide **18** was refluxed with acetic anhydride for 24 h, bis ester **19** was generated as the major product and was isolated after column chromatography in 68% yield. After attempting various base-hydrolysis procedures on **19**, it was found that the use of potassium carbonate in a MeOH/H_2O mixture effected a quantitative trans-esterification to give **20**. Finally, diol **20** was converted to the dichloride **4b** by treatment with thionyl chloride (64% yield). Stepwise conversion of **2b** to **4b** was thus made in an overall yield of 32%. It can be noticed, that **20** may be obtained more directly from bis *N*-oxide **18** using the same methodology, without isolating the intermediate bis ester **19** in 63% yield.

2.4. Application: synthesis of multidentate ligands

In the last part of this work, we used dibromide **4a** in the construction of architectures suitable for complexation of lanthanide ions (Schemes 7 and 8).

Alkylation of the secondary amine of di-*tert*-butyl iminodiacetate with **4a** was carried out in the presence of a mineral base to reach compound **5a** in excellent yield. Deprotection of the ester groups in aqueous 6 N HCl gave quantitatively the penta-acid **5b**. In preliminary experiments, the suitability of the **5b**·Eu^{3+} complex for protein labeling was investigated using Bovine Serum Albumin (BSA) as a model protein. Activation of the noncoordinated aromatic carboxylate function of **5b**·Eu^{3+} was achieved with EDCI·HCl in DMF and *N*-hydroxysuccinimide (NHS). In our experimental conditions, a labeling ratio (number of **5b**·Eu^{3+} per BSA) of 1.6 was established by MALDI-TOF mass spectrometry (See Experimental part).

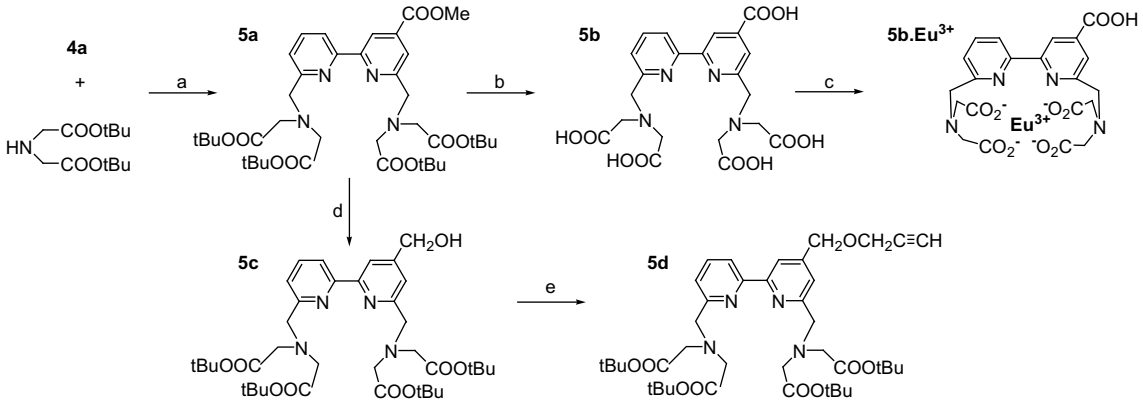


Scheme 8. Synthesis of cryptates **6a**·Na^+ and **6a**·Ln^{3+}. Reagents and conditions: (a) EtOH, reflux 24 h (65%), according to ref 53; (b) H_2SO_4, 120 °C, 2 h (88%) according to ref 53; (c) **4a**, Na_2CO_3, CH_3CN, reflux, 48 h, [reactants]=1.5.10^{-3} M (47%); (d) $LnCl_3 6H_2O$ (1.1 equiv), MeOH, reflux, 24 h.

Scheme 7 shows also the synthesis of acetylenic compound **5d**, suitable for 'click-chemistry'. Starting from **5a**, it was obtained in a two step sequence: i) selective reduction of the methyl ester group with $NaBH_4$ and ii) alkylation of the hydroxyl group with propargyl bromide.

The Lehn cryptand [N_2(bpy)$_3$] monofunctionalized by a methyl ester moiety **6a** is accessible by a stepwise procedure involving the synthesis of the intermediate macrocyclic bis(bipyridinediyl)diamine **23** and its subsequent bridging (Scheme 8).

The macrocycle **23** was obtained as previously described.[53] Condensation of the dibromo bipyridine fragment **21** with tosylamide monosodium salt in EtOH at reflux afforded the ditosylated compound **22** (65% yield) which was deprotected in concentrated H_2SO_4 by heating giving **23** in a 88% yield. Treatment of macrocyclic diamine **23** with dibromide **4a** was carried out in acetonitrile (reflux, 48 h) and in the presence of sodium carbonate as a base and



Scheme 7. Synthesis of open chain ligands **5**. Reagents and conditions: (a) Na_2CO_3, CH_3CN, reflux, 17 h (91%); (b) aqueous 6 N HCl, 90 °C, 72 h (100%); (c) $EuCl_3 \cdot 6H_2O$, 1.1 equiv, H_2O, rt, 24 h; (d) $NaBH_4$, MeOH, rt, 2 h (93%); (e) i) NaH, DMF, rt, ii) propargyl bromide, rt, 48 h (41%).

without using high-dilution techniques (reactant conditions 1.5×10^{-3} M). Cryptand **6a** was isolated as its NaBr complex after purification by column chromatography (47% yield).

2.5. Photophysical properties of the tethered trisbipyridine Eu(III) cryptate 6a·Eu^{3+}

The Ln^{3+} complexes (Ln=Eu, Gd) of the ligand **6a** were prepared by ion exchange from **6a**·Na^+. Sodium ion was displaced by simply refluxing for 24 h the sodium cryptate in the presence of a slight excess of $LnCl_3\cdot6H_2O$ in methanol solution. The resulting **6a**·Ln^{3+} complexes were isolated by precipitation with diethyl ether.

At room temperature **6a**·Eu^{3+} cryptate is luminescent in the red domain when excited into the lowest-energy LC absorption band (306 nm), indicating that the energy is absorbed by the surrounding ligand and efficiently transferred to the chelated Eu^{3+} ion. Sensitized Eu^{3+} emission is observed corresponding to $^5D_0\rightarrow{}^7F_J$ (J=0–4) transitions, the dominant band in the emission spectrum is the transition $^5D_0\rightarrow{}^7F_2$ at 617 nm (Fig. 1).

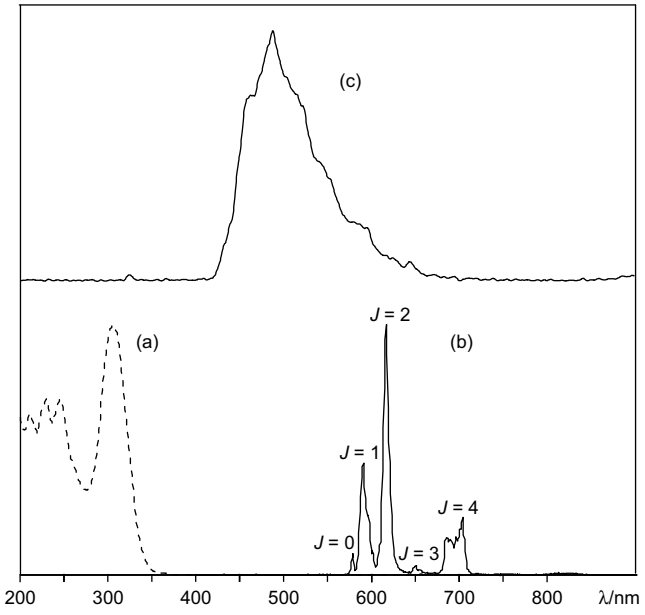


Figure 1. (a) excitation (250–450 nm, λ_{em}=617 nm) and (b) emission (λ_{exc}=306 nm) spectra of the **6a**·Eu^{3+} cryptate in water at 298 K. The emission bands arise from the $^5D_0\rightarrow{}^7F_J$ transitions; the J values are shown on the spectrum. (c) emission spectrum (λ_{exc}=306 nm) of the **6a**·Gd^{3+} cryptate in water/glycerol (7/3) at 77 K.

The luminescence lifetimes (τ) and quantum yields (Φ) were measured under various experimental conditions. The results are summarized in Table 1 where some pertinent data previously obtained for the corresponding methyl ester disubstituted derivative [N_2(bpy)$_3$(CO_2Me)$_2$Eu] (**6b**·Eu^{3+}) are also shown for comparison purposes. The value of the luminescence lifetime in H_2O solution at 298 K (0.36 ms) is diagnostic of an aquo europium complex. By using the well-established isotope effect and the Horrocks equation[54] the hydration number (i.e., the number of coordinated water molecules) is determined to be 2.3. After correction for the weaker effect of outer-sphere water molecules,[55] this value is reduced to 2.1. We can also notice that, in D_2O solution, the lifetime of the $^5D_0$ level is temperature independent, indicating that thermally activated radiationless decay paths play any role in the decay of the metal-centered emitting state. The modest value measured for the emission quantum yield (1.8%) is attributed to nonradiative deactivation via the O–H vibrations of the two inner sphere water molecules and to the presence of low energy ligand-to-metal charge-transfer excited state (LMCT), as commonly observed for europium complexes with bpy-type cryptands.[15,56] However, as reported in the TRACE® technology,[57] the addition of fluoride ions may be used for enhancing the luminescence efficiency of europium cryptate. When **6a**·Eu^{3+} (10 μM) is incubated at room temperature in the presence of a saturating anion concentration (0.4 M, F^-), a 4-fold increase of the total emission intensity is observed.

For **6a**·Gd^{3+}cryptate, ligand excitation at 77 K and time-resolved measurement at 77 K reveal a broad emission band extending from 400 to 750 nm, with a maximum at 487 nm, which is assigned to the phosphorescence of the coordinated cryptand (Fig. 1). The lowest excited state ($^6P_{7/2}$) of the Gd^{3+} ion lies above 31,000 cm^{-1},[58] and thus cannot accept energy from the ligand; as a result, **6a**·Gd^{3+} cryptate displays ligand-centered photophysical process only. The position of the lowest cryptand-centered excited triplet state was located at 461 nm (21,700 cm^{-1}), determined from the highest energy phosphorescence feature of the spectrum (Fig. 1) and the corresponding lifetime is 4.30 ms. This lifetime is substantially shorter than that measured for parent sodium cryptate (second timescale), indicating a great perturbation of the electron density of the macrobicycle by the metal ion and suggesting a strong interaction Ln^{3+}-ligand.

As one can see from data gathered in Table 1, the luminescence properties (τ, Φ) and the estimated hydration state of the metal in **6a**·Eu^{3+} are similar to those reported for the corresponding disubstituted europium cryptate **6b**·Eu^{3+}.[59] The small differences observed are close to the experimental error. The modification in the triplet state energy is too small to alter the efficiency of the energy transfer from the ligand to the europium ion. Clearly, like its disubstituted analogue, **6a**·Eu^{3+} may be used as a luminescent marker for HTRF assays but without the potential drawback of the occurrence of undesirable intra- and intermolecular cross-linking in bioconjugation reactions.

3. Conclusion

After attempting several methodologies generally employed for the synthesis of unsymmetrical 2,2′-bpy derivatives, it was found that a modified Negishi cross-coupling reaction was the most effective for the multigram preparation of 4-carbomethoxy-6,6′-dimethyl-2,2′-bipyridine. This bpy derivative is a versatile building block for a wealth of further transformations, as demonstrated by some examples. Subsequent transformations of the aromatic ester

Table 1
Absorption maxima (λ_{max} in nm), absorption coefficients (ϵ_{max} in $M^{-1}\,cm^{-1}$), luminescence lifetimes (τ in ms), quantum yields (Φ%), hydration state (q) and triplet state energies (E_T in cm^{-1}) for **6a**·Eu^{3+} and its corresponding disubstituted analogue **6b**·Eu^{3+}[a]

	λ_{max}	ϵ_{max}	$\tau_H^{298\,K}$	$\tau_{H,F^-}^{298\,K}$	$\tau_D^{298\,K}$	$\tau_D^{77\,K}$	$\Phi_H^{298\,K}$	$\Phi_{H,F^-}^{298\,K}$	q[b]	E_T[c]
6a·Eu^{3+}	306	27,500	0.36	1.17	1.70	1.70	1.8	7.0	2.1	21,700
6b·Eu^{3+}[d]	306	26,400	0.34	—	1.50	1.50	2	—	2.2	21,800

[a] Data obtained in aerated water, H_2O (H) or D_2O (D) solutions; in the absence or in the presence of 0.4 M KF (F^-).
[b] Number of coordinated H_2O molecules calculated using the equation q=1.11 ($1/\tau_H-1/\tau_D-0.31$).[55]
[c] From phosphorescence spectra at 77 K of **6a**·Gd^{3+} and **6b**·Gd^{3+} complexes.
[d] Data from ref 59.

group to various functionalities were successfully carried out, opening avenues for further grafting of biological material by classical methods or 'click chemistry'. Using the methods described here, it will be possible to synthesize 4-subsituted 2,2′-bipyridine derivatives with spacers of any desired length and functionalized tails. We have also shown that the methyl groups at the 6,6′-positions can be simply converted into bromomethyl and chloromethyl groups, which are common moieties for the development of preorganized bpy-containing architectures. As first examples in the direction of luminescent lanthanide complexes, an open chain ligand based on one 2,2′-bipyridine unit and a cryptand based on three 2,2′-bipyridine units were synthesized. The preliminary labeling experiments and photophysical studies of their corresponding Eu(III) complexes nominate these ligands as candidates for luminescent labeling of biological material.

4. Experimental

4.1. General methods

Reactions requiring an inert atmosphere were run under Argon. THF and Et_2O were freshly distilled from Na/benzophenone, acetonitrile was freshly distilled from P_2O_5, and CH_2Cl_2 was freshly distilled from CaH_2. Butyllithium was titrated before use with menthol, using 1,10-phenanthrolin as indicator. Thin-layer chromatography was performed on Merck silica or alumina plates with a fluorescence indicator. TLC spots were visualised by irradiation with UV light or by exposure to iodine vapours (R_f values refer to relative mobilities on TLC plates). Column chromatography was carried out on silica gel (Merck, 60–200 μm, porosity 60 Å) and on alumina (Macherey-Nagel, activity IV, 50–200 μm).

Melting points were taken with a Büchi mel-temp apparatus. Infrared spectra were recorded on a Perkin–Elmer 883 spectrophotometer. Samples were prepared as KBr pellets (solid sample) or applied to NaCl plates (liquid sample). Selected characteristic absorbances are reported in cm^{-1}. Proton magnetic resonance spectra (^1H NMR) were recorded at 250 or 300 MHz on a Bruker AC 250 MHz or Bruker Avance 300 MHz spectrometer and are reported as follows: chemical shift δ in parts per million (multiplicity, number of protons, coupling constant J in hertz). Residual protic solvent was used as the internal reference, setting chloroform to δ 7.26. Carbon magnetic resonance spectra (^{13}C NMR) were recorded at 75 MHz on a Bruker Avance 300 MHz spectrometer. Chemicals shifts are quoted in parts per million, referenced to the appropriate solvent peak, taking chloroform as δ 77.0. Electrospray (ES) mass spectra were obtained on a Perkin–Elmer SCIEX API 100 and Waters Q-TOF spectrometers. Elemental analyses were carried out by the 'Service d'Analyse', Laboratoire de Chimie de Coordination (Toulouse). Absorption measurements were done with a Hewlett Packard 8453 temperature controlled spectrophotometer.

The Eu(III) and Gd(III) luminescence emission and excitation spectra were acquired using a LS-50B Perkin–Elmer spectrofluorimeter equipped with a Hamamatsu R928 photomultiplier tube and with the low-temperature accessory No. L2250136. Lifetimes τ (uncertainty$\leq$5%) are the average values from at least ten separate measurements covering two or more lifetimes. The phosphorescence decay curves were fitted by an equation of the form $I(t)=I(0)\exp(-t/\tau)$ using a curve-fitting program. The luminescence quantum yields (uncertainty±15%) were determined by the method described by Haas and Stein,[60] using as standard $[Ru(bpy)_3]^{2+}$ in aerated water (Φ=0.028)[61] and corrected for the refractive index of the solvent.

The following compounds were prepared as described in the literature: 2-Bromo-6-methylpyridine **6**,[33] 2-tributylstannyl-6-methylpyridine **12**,[41] 6,6-Bis (bromomethyl)-2,2′-bipyridine **20**,[62] macrocyclic diamines **21** and **22**.[53]

4.2. 3-Cyano-6-methyl-2-oxo-1,2-dihydro-pyridine-4-carboxylic acid ethyl ester (7)

Absolute ethyl alcohol (300 mL) was placed in a round-bottomed flask and purged with argon. Sodium (13.5 g, 0.59 mol) was added per portions over a period of 1 h and the solution was stirred overnight. A mixture of diethyl oxalate (68 mL, 0.50 mol) and acetone (37 mL, 0.50 mol) was added dropwise, observing the precipitation of a solid turning from white to yellow. The resulting slurry was stirred for 3 h at rt. After this time, cyanoacetamide (42.0 g, 0.50 mol) was added and the mixture was heated to 80 °C for 6 h. Ethanol was removed and the solid residue was dried under vacuum. The resulting solid was dissolved in boiling water (135 mL), and then acetic acid (10 mL) was added, whereupon a precipitate was formed. The orange-coloured precipitate was collected by filtration and dried under vacuum to give **7** (70.3 g, 0.34 mol, yield 68%). Mp: 214–215 °C. (litt[63] mp 213 °C). R_f (Silica gel, 6% MeOH in CH_2Cl_2): 0.50. IR υ_{max}: 3444, 2840, 2228 (C≡N), 1733 (C=O ester), 1646 (C=O amide), 1251. ^1H NMR (250 MHz, DMSO-d_6) δ: 1.31 (t, 3H, J=7.1), 2.32 (s, 3H), 4.35 (q, 2H, J=7.1), 6.55 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 14.1 (CH_3), 19.7 (CH_3), 63.0 (CH_2), 99.4 (Cq), 104.8 (CH), 115.1 (Cq), 147.6 (Cq), 154.6 (Cq), 161.5 (Cq), 163.2 (Cq). MS (ESI^+): m/z (%) 207 (15) $[M+H]^+$, 229 (100) $[M+Na]^+$, 245 (43) $[M+K]^+$.

4.3. 6-Methyl-2-oxo-1,2-dihydropyridine-4-carboxylic acid (8)

Cyanoester **7** (15.0 g, 72.8 mmol) was added to 140 mL of 6 M aq HCl, and the resulting mixture was heated to a gentle reflux with stirring for 24 h. The hot reaction mixture was poured onto approx. 250 g of ice. A fine beige solid precipitated, which was collected by vacuum filtration, washed with 30 mL of ice water, and dried under vacuum, to give acid **8** (10.1 g, 66.0 mmol, yield 91%) as a pale beige solid. Mp: >260 °C dec. R_f (Silica gel, CH_2Cl_2/MeOH 1:1): 0.40. IR υ_{max}: 3424, 2930, 1718 (C=O acid), 1641 (C=O amide), 1444, 1234. ^1H NMR (250 MHz, DMSO-d_6) δ: 2.23 (s, 3H), 3.59 (br s, exchangeable protons), 6.37 (s, 1H), 6.61 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 19.2 (CH_3), 103.2 (CH), 118.0 (CH), 143.1 (Cq), 147.2 (Cq), 163.7 (Cq), 166.6 (Cq). MS (ESI^-): m/z (%) 152 (100) $[M-H]^-$.

4.4. 2-Chloro-6-methylpyridine-4-carboxylic acid methyl ester (9)

To a stirred solution of $POCl_3$ (10 mL, 107 mmol) was added acid **8** (2.37 g, 15.5 mmol), and the resulting solution was heated to reflux for 18 h. The excess $POCl_3$ was distilled off under reduced pressure, the residue was cooled to 0 °C, methanol (20 mL) was added dropwise, and the resulting mixture was stirred for 24 h at rt. The reaction mixture was neutralized with solid $NaHCO_3$, and partitioned between H_2O (30 mL) and EtOAc (30 mL). The aqueous layer was extracted with EtOAc, (2×30 mL) and the combined organic layers were treated with active carbon, then filtered through Celite, dried (Na_2SO_4), and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography over silica gel (CH_2Cl_2), to give ester **9** (2.40 g, 12.9 mmol, yield 83%) as a white solid. Mp: 61 °C. R_f (Silica gel, CH_2Cl_2): 0.66. IR υ_{max}: 3092, 2961, 1736 (C=O), 1557, 1429, 1303, 1226, 1166. ^1H NMR ($CDCl_3$, 300 MHz) δ: 2.60 (s, 3H), 3.95 (s, 3H), 7.63 (s, 1H), 7.68 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 24.1 (CH_3), 52.8 (CH_3), 120.8 (Cq), 121.2 (Cq), 140.3 (Cq), 151.3 (Cq), 160.5 (Cq), 164.5 (Cq). MS (ESI^+): m/z (%) 186 (100) $[M+H]^+$, 208 (35) $[M+Na]^+$.

4.5. 2-Methyl-6-(trimethylsilyl)-pyridine (11)

To a stirred solution of bromopyridine **6** (1.00 g, 5.81 mmol) in anhydrous THF (10 mL), at −78 °C, was added, dropwise over

15 min, *n*BuLi (1.3 M in hexanes, 4.9 mL, 6.4 mmol). The resulting red solution was stirred at −78 °C for 3 h, followed by the dropwise addition over 10 min. of TMSCl (900 μL, 7.00 mmol). The resulting mixture was allowed to warm up to rt, and stirring was continued for 18 h. The reaction mixture was quenched by addition of H_2O (12 mL), and extracted with Et_2O (3×15 mL). The combined organic layers were dried (Na_2SO_4), and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography over silica gel (petroleum ether/Et_2O 90:10 then 80:20), to give pyridylsilane **11** (530 mg, 3.21 mmol, yield 55%) as a pale yellow liquid. IR υ_{max}: 3053, 2957, 1574, 1444, 1248, 1141. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.33 (s, 9H), 2.59 (s, 3H), 7.04 (dd, 1H, J=7.8, 0.75), 7.31 (d, 1H, J=7.2), 7.46 (t, 1H, J=7.5). ^{13}C NMR (75 MHz, $CDCl_3$) δ: −1.7 (CH_3), 24.9 (CH_3), 122.3 (CH), 125.7 (CH), 133.9 (CH), 158.3 (Cq), 167.6 (Cq). MS (DCI/NH_3): m/z (%) 166 (31) $[M+H]^+$.

4.6. 4-Carbomethoxy-6,6′-dimethyl-2,2′-bipyridine (1)

4.6.1. Via Suzuki–Miyaura cross-coupling reaction

A mixture of pyridyl chloride **9** (0.63 g, 3.4 mmol), 2-pyridylboronic ester **10** (1.15 g, 4.08 mmol), $PdCl_2(PPh_3)_2$ (119 mg, 0.17 mmol), anhydrous K_3PO_4 (1.5 g, 7 mmol), and CuI (325 mg, 1.7 mmol) in anhydrous DMF (40 mL) was heated to 100 °C under Argon for 6 h. The reaction mixture was warmed up to rt, diluted with diethyl ether (50 mL), washed with water (2×20 mL), dried over $MgSO_4$ and the solvent was removed under reduced pressure. The solid residue was purified by flash chromatography (alumina, petroleum ether/Et_2O 90:10) to give bipyridine **1** (370 mg, 1.53 mmol, yield 45%) as a white solid.

4.6.2. Via Stille cross-coupling reaction

To a stirred degassed solution of pyridyl chloride **9** (0.63 g, 3.4 mmol) and of pyridylstannane **12** (1.53 g, 4.0 mmol) in toluene (20 mL) was added $Pd(PPh_3)_4$ (200 mg, 0.17 mmol), and the resulting mixture was heated to reflux for 24 h. The reaction mixture was filtered over Celite and concentrated aqueous HCl (20 mL) was added to the residue, and the resulting mixture was washed with CH_2Cl_2 (3×20 mL). The aqueous layer was neutralized with solid $NaHCO_3$, and extracted with CH_2Cl_2 (3×20 mL). The latter organic layers were dried (Na_2SO_4) and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (alumina, petroleum ether/Et_2O 90:10) to give bipyridine **1** (540 mg, 2.23 mmol, yield 66%) as a white solid.

4.6.3. Via Negishi cross-coupling reaction

A solution of *n*-butyllithium (1.5 M in hexanes, 45 mL, 67.5 mmol) was slowly added to a stirred solution of bromopicoline **6** (10.08 g, 58.6 mmol) in anhydrous THF (50 mL) at −78 °C under Ar in a 500-mL glass reactor. During the addition the temperature was kept at −60 °C maximum. The resulting red solution was stirred for 30 min at −78 °C, followed by the dropwise addition of anhydrous $ZnCl_2$ (it was used after fusion by flame-drying under reduced pressure) (9.14 g, 67.1 mmol) in THF (150 mL). The resulting mixture was warmed up to rt, and stirring was continued for 1 h. In a separate 1-L glass reactor, DIBALH (1.0 M in hexanes, 4 mL, 4 mmol) was added to a stirred solution of $PdCl_2(PPh_3)_2$ (1.46 g, 2.08 mmol) in anhydrous THF (150 mL), at rt under Argon. The yellow solution turning black was stirred for 30 min before the addition of a solution of pyridyl chloride **9** (7.04 g, 37.9 mmol) in THF (50 ml). The resulting solution was stirred at rt for 10 min. The pyridylzinc chloride (**13**) solution prepared above was then added via canula, and the resulting yellow mixture was heated at reflux until complete consumption of compound **9** was observed by TLC monitoring (4 h). The reaction mixture was cooled to rt, then satd aq $NaHCO_3$ solution (400 mL) was added. The aqueous layer was extracted with Et_2O (3×250 mL) and the organic extracts were concentrated under pressure to give a residue, which was purified by flash chromatography over alumina (petroleum ether/Et_2O 90:10), to give bipyridine **1** (7.60 g, 31.4 mmol, yield 83%) as a white solid.

*4.6.4. Compound **1***

Mp: 122–123 °C (litt.[24] 116–117 °C). R_f (alumina, petroleum ether/Et_2O 90:10): 0.50. IR υ_{max}: 2958, 1733 (C=O), 1571, 1429, 1340, 1255. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.64 (s, 3H), 2.69 (s, 3H), 3.97 (s, 3H), 7.18 (d, 1H, J=7.8), 7.69 (t, 1H, J=7.8), 7.71 (s, 1H), 8.19 (d, 1H, J=7.8), 8.71 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 24.6 (CH_3), 52.5 (CH_3), 117.7 (CH), 118.3 (CH), 122.2 (CH), 123.5 (CH), 137.0 (CH), 138.6 (Cq), 155.1 (Cq), 157.1 (Cq), 158.1 (Cq), 159.0 (Cq), 166.2 (Cq). MS (DCI/NH_3): m/z (%) 243 (100) $[M+H]^+$. Anal. Calcd for $C_{14}H_{14}N_2O_2$: C, 69.41; H, 5.82; N, 11.56. Found: C, 69.25; H, 5.75; N, 11.44.

4.7. 6,6′-Dimethyl-2,2′-bipyridine-4-acetic acid (2a)

A solution of K_2CO_3 (28.4 mg, 0.21 mmol) in water (2.5 mL) was added to a stirred solution of ester **1** (50 mg, 0.21 mmol) in methanol (5.5 mL). The solution was heated to 80 °C for 0.5 h. The solvent was removed under reduced pressure, and the aqueous layer was acidified with 0.5 N HCl to pH 3.5–4.0. A white precipitate separated upon cooling. The solid was collected by centrifugation and dried under reduced pressure to give **2a** (47 mg, 0.21 mmol, yield 100%). Mp: >200 °C dec. IR υ_{max}: 2928, 1711 (C=O), 1592, 1571. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.62 (s, 3H), 2.66 (s, 3H), 7.31 (d, 1H, J=7.6), 7.77 (d, 1H, J=0.9), 7.81 (t, 1H, J=7.7), 8.13 (d, 1H, J=7.8), 8.58 (d, 1H, J=0.9). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 24.2 (CH_3), 24.35 (CH_3), 119.2 (CH), 120.0 (CH), 123.8 (CH), 125.1 (CH), 139.05 (CH), 141.5 (Cq), 156.0 (Cq), 157.7 (Cq), 159.5 (Cq), 160.55 (Cq), 168.35 (Cq). MS (DCI^+/CH_4): m/z (%) 229.1 (100) $[M+H]^+$, 257.1 (17) $[M+C_2H_5]^+$.

4.8. 6,6′-Dimethyl-4-hydroxymethyl-2,2′-bipyridine (2b)

To a solution of ester **1** (4.84 g, 20.0 mmol) in anhydrous ethanol (40 mL) was added $NaBH_4$ as a solid (1.90 g, 50.2 mmol) with stirring at rt under Argon. The resulting mixture was further stirred for 16 h at rt. A solution of satd aq NH_4Cl (20 mL) was added, and the resulting mixture was partitioned between CH_2Cl_2 (50 mL) and H_2O (50 mL). The aqueous layer was extracted with CH_2Cl_2 (2×50 mL), the combined organic layers were dried (Na_2SO_4) and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography over silica gel (CH_2Cl_2/MeOH 98:2 then 90:10), to give alcohol **2b** (3.51 g, 16.4 mmol, yield 82%) as a white solid. Mp: 72–74 °C. IR υ_{max}: 3268, 2919, 1589, 1569, 1460, 1417, 1069, 803. ^{1}H NMR (250 MHz, $CDCl_3$) δ: 2.64 (s, 3H), 2.67 (s, 3H), 4.72 (s, 2H), 7.13 (s, 1H), 7.17 (d, 1H, J=7.6), 7.69 (t, 1H, J=7.8), 8.08 (s, 1H), 8.13 (d, 1H, J=7.6). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 24.52 (CH_3), 24.54 (CH_3), 63.6 (CH_2), 115.8 (CH), 118.5 (CH), 120.5 (CH), 123.2 (CH), 137.1 (CH), 151.1 (Cq), 155.8 (Cq), 156.0 (Cq), 157.9 (Cq), 158.2 (Cq). MS (ESI^+): m/z (%) 215.3 (100) $[M+H]^+$, 237.2 (29) $[M+Na]^+$. Anal. Calcd for $C_{13}H_{14}N_2O$: C, 72.87; H, 6.59; N, 13.07. Found: C, 72.73; H, 6.30; N, 12.89.

4.9. *N*-(2-Aminoethyl)-6,6′-dimethyl-2,2′-bipyridine-4-carboxamide (2c)

Ester **1** (100 mg, 0.41 mmol) was added per portions to ethylenediamine (759 mg, 12.6 mmol) at rt. The mixture was stirred at rt for 24 h, observing the consumption of the starting material on TLC. Excess of ethylenediamine was eliminated under high vacuum to give **2c** as a grey solid (111 mg, 0.41 mmol, yield 100%). Mp=150–151 °C. IR υ_{max}: 3363, 3278, 3046, 2908, 2860, 1651 (C=O), 1563. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.63 (s, 3H), 2.67 (s, 3H), 2.97 (t, 2H, J=5.8), 3.55 (q, 2H, J=5.8), 7.18 (d, 1H, J=7.8), 7.60 (s, 1H), 7.70 (t, 1H,

J=7.8), 8.21 (d, 1H, J=7.8), 8.44 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 24.55 (CH_3), 24.6 (CH_3), 41.2 (CH_2), 42.6 (CH_2), 114.7 (CH), 118.4 (CH), 121.0 (CH), 123.6 (CH), 137.2 (CH), 142.8 (Cq), 155.1 (Cq), 156.6 (Cq), 157.9 (Cq), 159.2 (Cq), 166.35 (Cq). MS (ESI$^+$): m/z (%) 271.3 (100) $[M+H]^+$.

4.10. 4-[6-(*tert*-Butyldimethylsilanyloxy)hexyloxymethyl]-6,6′-dimethyl-2,2′-bipyridine (14)

To a stirred solution of alcohol **2b** (146 mg, 0.68 mmol) in anhydrous DMF (5 mL) was added sodium hydride (35.2 mg, 1.47 mmol) in one portion under Argon. The resulting mixture was heated to 60 °C for 1 h, followed by the addition of a solution of (6-bromohexyloxy)-*tert*-butyldimethylsilane (419 mg, 1.42 mmol) in DMF (3 mL). The resulting mixture was stirred at 60 °C for 24 h. After cooling down to rt, the reaction mixture was partitioned between H_2O (10 mL) and EtOAc (50 mL). The organic layer was washed with H_2O (3×10 mL), dried ($MgSO_4$), and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (petroleum ether/EtOAc 90:10), to give ether **14** (166 mg, 0.39 mmol, yield 57%) as a colorless oil. R_f (silica gel, petroleum ether/EtOAc 90:10): 0.16. IR υ_{max}: 2930, 2857, 1586, 1570, 1462, 1419, 1254, 1098, 836. ^{1}H NMR (250 MHz, $CDCl_3$) δ: 0.04 (s, 6H), 0.89 (s, 9H), 1.38–1.67 (m, 8H), 2.64 (s, 6H), 3.52 (t, 2H, J=6.6), 3.61 (t, 2H, J=6.6), 4.57 (s, 2H), 7.16 (d, 1H, J=7.3), 7.20 (s, 1H), 7.69 (t, 1H, J=7.6), 8.09 (s, 1H), 8.17 (d, 1H, J=7.6). ^{13}C NMR (75 MHz, $CDCl_3$) δ: −5.3 (CH_3), 18.3 (Cq), 24.6 (CH_3), 25.6 (CH_2), 25.92 (CH_3), 25.94 (CH_2), 29.7 (CH_2), 32.75 (CH_2), 63.1 (CH_2), 71.0 (CH_2), 71.5 (CH_2), 116.4 (CH), 118.2 (CH), 121.1 (CH), 123.0 (CH), 136.9 (CH), 148.8 (Cq), 155.85 (Cq), 155.9 (Cq), 157.7 (Cq), 158.1 (Cq). MS (FAB$^+$, MNBA): m/z (%) 429.2 (100) $[M+H]^+$.

4.11. 6-(6,6′-Dimethyl-[2,2′]bipyridinyl-4-ylmethoxy)-hexan-1-ol (3a)

To a stirred solution of silyl ether **14** (118.6 mg, 0.277 mmol) in anhydrous THF (5 mL), at 0 °C under Argon, was added dropwise tetra-*n*-butylammonium fluoride (1 M in THF, 3 mL, 3 mmol). The resulting solution was stirred at 0 °C for 20 min, then warmed up to rt, and stirring was continued for 3 h. The reaction mixture was partitioned between H_2O (10 mL) and CH_2Cl_2 (10 mL), and the aqueous layer was extracted with CH_2Cl_2 (3×10 mL). The combined organic layers were dried (Na_2SO_4) and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography over alumina (CH_2Cl_2/MeOH 95:5), to give alcohol **3a** (70 mg, 0.222 mmol, yield 80%) as a colorless oil. IR υ_{max}: 3368, 2932, 2858, 1587, 1569, 1420, 1115. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.32–1.63 (m, 8H), 2.56 (s, 6H,), 3.40 (s, 1H), 3.46 (t, 2H, J=6.5), 3.57 (t, 2H, J=6.5), 4.50 (s, 2H), 7.08 (d, 1H, J=7.5), 7.11 (s, 1H), 7.62 (t, 1H, J=7.8), 8.03 (s, 1H), 8.10 (d, 1H, J=7.8). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 24.6 (CH_3), 25.5 (CH_2), 25.9 (CH_2), 29.6 (CH_2), 32.6 (CH_2), 62.8 (CH_2), 70.9 (CH_2), 71.5 (CH_2), 116.5 (CH), 118.3 (CH), 121.2 (CH), 123.0 (CH), 137.0 (CH), 148.8 (Cq), 155.8 (Cq), 156.0 (Cq), 157.8 (Cq), 158.1 (Cq). MS (ESI$^+$): m/z (%) 315.2 (100) $[M+H]^+$, 337.35 (36) $[M+Na]^+$. Anal. Calcd for $C_{19}H_{26}N_2O_2$: C, 72.58; H, 8.33; N, 8.91. Found: C, 72.35; H, 8.23; N, 8.77.

4.12. 4-(6-Bromohexyloxymethyl)-6,6′-dimethyl-2,2′-bipyridine (3b)

To a stirred solution of alcohol **2b** (411 mg, 1.92 mmol) in anhydrous THF (5 mL) was added under Argon sodium hydride (75 mg, 3.1 mmol). The resulting mixture was heated to reflux for 1.5 h, followed by addition of 1,6-dibromohexane (1.22 g, 5.0 mmol). The resulting solution was heated to reflux under Argon for 20 h. After cooling to rt, the reaction mixture was diluted with EtOAc (50 mL), washed with H_2O (3×15 mL), dried (Na_2SO_4), and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography over silica gel (CH_2Cl_2/MeOH 97:3), to give bromide **3b** (361 mg, 0.96 mmol, yield 50%) as a yellow oil. R_f (alumina, CH_2Cl_2/petroleum ether 60:40): 0.48. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.41–1.45 (m, 4H), 1.62–1.67 (m, 2H), 1.83–1.88 (m, 2H), 2.57 (s, 6H), 3.34 (t, 2H, J=6.8), 3.46 (t, 2H, J=6.4), 4.50 (s, 2H), 7.12 (d, 1H, J=7.6), 7.16 (s, 1H), 7.65 (t, 1H, J=7.7), 8.01 (s, 1H), 8.17 (d, 1H, J=7.8). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 24.7 (CH_3), 25.4 (CH_2), 28.0 (CH_2), 29.5 (CH_2), 32.7 (CH_2), 33.8 (CH_2), 70.8 (CH_2), 71.5 (CH_2), 116.5 (CH), 118.3 (CH), 121.2 (CH), 123.1 (CH), 137.0 (CH), 148.8 (Cq), 155.9 (Cq), 156.0 (Cq), 157.8 (Cq), 158.2 (Cq). MS (ESI$^+$): m/z (%) 377.3 (100) $[M+H]^+$, 379.3 (94) $[M+H]^+$. Anal. Calcd for $C_{19}H_{25}N_2OBr$: C, 60.48; H, 6.68; N, 7.42. Found: C, 60.47; H, 7.23; N, 7.81.

4.13. 2-[6-(6,6′-Dimethyl-[2,2′]bipyridinyl-4-ylmethoxy)-hexyl]-isoindole-1,3-dione (15)

To a stirred solution of bromide **3b** (172 mg, 0.46 mmol) in DMF (5 mL) was added potassium phthalimide (168 mg, 0.91 mmol), and the resulting mixture was heated to 100 °C for 5 h. The reaction mixture was diluted with 50 mL of EtOAc, washed with H_2O (4×10 mL), dried (Na_2SO_4), and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography over alumina (petroleum ether/Et_2O 50:50) to give phthalimide **15** (160 mg, 0.36 mmol, yield 78%) as a white solid. Mp: 155 °C. IR υ_{max}: 2935, 2859, 1772 (C═O), 1713 (C═O), 1585, 1573, 1396, 1370, 1115. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.38–1.46 (m, 4H), 1.60–1.75 (m, 4H), 2.63 (s, 6H), 3.51 (t, 2H, J=6.6), 3.69 (t, 2H, J=7.2), 4.55 (s, 2H), 7.14 (d, 1H, J=7.5), 7.18 (s, 1H), 7.65–7.71 (m, 3H), 7.82–7.85 (m, 2H), 8.08 (s, 1H), 8.17 (d, 1H, J=7.8). ^{13}C NMR (75 MHz, $CDCl_3$,) δ: 24.6 (CH_3), 25.7 (CH_2), 26.7 (CH_2), 28.5 (CH_2), 29.5 (CH_2), 37.9 (CH_2), 70.9 (CH_2), 71.4 (CH_2), 116.5 (CH), 118.3 (CH), 121.1 (CH), 123.0 (CH), 123.1 (CH), 132.1 (Cq), 133.8 (CH), 136.9 (CH), 148.8 (Cq), 155.8 (Cq), 155.9 (Cq), 157.8 (Cq), 158.1 (Cq), 168.4 (Cq). MS (ESI$^+$): m/z (%) 444.2 (100) $[M+H]^+$.

4.14. 6-(6,6′-Dimethyl-[2,2′]bipyridinyl-4-ylmethoxy)-hexylamine (3c)

To a stirred solution of phthalimide **15** (120.2 mg, 0.27 mmol) in methanol (3 mL) was added hydrazine monohydrate (50 μl, 1.0 mmol), and the resulting mixture was stirred at rt for 3 h. The reaction mixture was partitioned between H_2O (5 mL) and CH_2Cl_2 (10 mL), and the aqueous layer was extracted with CH_2Cl_2 (3×5 mL). The combined organic layers were dried (Na_2SO_4) and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography over alumina (CH_2Cl_2/MeOH 98:2), to give amine **3c** (82.2 mg, 0.26 mmol, yield 96%) as a yellow oil. IR υ_{max}: 3368, 2931, 2858, 1609, 1585, 1573, 1462, 1420, 1115. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.30–1.77 (m, 8H), 2.63 (s, 6H), 2.70 (t, 2H, J=6.75), 3.52 (t, 2H, J=6.5), 4.57 (s, 2H), 7.15 (d, 1H, J=7.8), 7.19 (s, 1H), 7.68 (t, 1H, J=7.7), 8.10 (s, 1H), 8.18 (d, 1H, J=7.8). ^{13}C NMR (75 MHz, $CDCl_3$,) δ: 24.7 (CH_3), 26.1 (CH_2), 26.7 (CH_2), 29.7 (CH_2), 33.5 (CH_2), 42.1 (CH_2), 71.0 (CH_2), 71.5 (CH_2), 116.5 (CH), 118.3 (CH), 121.2 (CH), 123.1 (CH), 137.0 (CH), 148.9 (Cq), 155.9 (Cq), 156.0 (Cq), 157.8 (Cq), 158.2 (Cq). MS (ESI$^+$): m/z (%) 314.4 (100) $[M+H]^+$. Anal. Calcd for $C_{19}H_{27}N_3O\cdot 1H_2O$: C, 68.85; H, 8.82; N, 12.68. Found: C, 68.58; H, 9.05; N, 12.50.

4.15. 2-Bromo-*N*-[6-(6,6′-dimethyl-[2,2′]bipyridinyl-4-ylmethoxy)-hexyl]-acetamide (3d)

To a stirred solution of amine **3c** (141 mg, 0.45 mmol) in anhydrous CH_2Cl_2 (5 mL) was added under Argon *N*-methylmorpholine

(132 μL, 1.2 mmol). The resulting solution was cooled down to 0 °C, followed by the addition of bromoacetyl chloride (45 μL, 0.54 mmol). The resulting solution was stirred at rt under Argon for 18 h. The reaction mixture was partitioned between H_2O (10 mL) and CH_2Cl_2 (10 mL), and the aqueous layer was extracted with CH_2Cl_2 (3×5 mL). The combined organic layers were dried (Na_2SO_4) and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography over alumina (CH_2Cl_2/MeOH 99:1), to give compound **3d** (140 mg, 0.322 mmol, yield 72%) as a yellow oil. IR υ_{max}: 2931, 2858, 1661, 1567, 1420, 1113. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.37–1.48 (m, 4H), 1.57 (p, 2H, J=7.1), 1.67 (p, 2H, J=7.1), 2.64 (s, 6H), 3.29 (q, 2H, J=6.7), 3.53 (t, 2H, J=6.4), 3.87 (s, 2H), 4.57 (s, 2H), 7.17 (d, 1H, J=7.6), 7.19 (s, 1H), 7.70 (t, 1H, J=7.7), 8.11 (s, 1H), 8.19 (d, 1H, J=7.9). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 24.0 (CH_3), 24.3 (CH_3), 25.8 (CH_2), 26.6 (CH_2), 29.2 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 40.1 (CH_2), 71.0 (CH_2), 71.2 (CH_2), 117.4 (CH), 119.3 (CH), 121.9 (CH), 123.9 (CH), 137.8 (CH), 150.6 (Cq), 153.9 (Cq), 154.3 (Cq), 157.8 (Cq), 157.9 (Cq), 165.2 (Cq). MS (ESI$^+$): m/z (%) 434.3 (97) $[M+H]^+$, 436.3 (100) $[M+H]^+$. Anal. Calcd for $C_{21}H_{28}N_3O_2Br$: C, 58.07; H, 6.50; N, 9.67. Found: C, 58.34; H, 6.55; N, 9.52.

4.16. 1-[6-(6,6′-Dimethyl-[2,2′]bipyridinyl-4-ylmethoxy)-hexyl]-pyrrole-2,5-dione (3e)

DEAD (37 μL, 0.235 mmol) was added dropwise to a stirred solution of alcohol **3a** (60 mg, 0.191 mmol), maleimide (22.6 mg, 0.233 mmol) and PPh_3 (61 mg, 0.233 mmol) in THF (2 mL) at 0 °C under Argon atmosphere. The reaction mixture was stirred at 0 °C for 12 h and then was quenched by addition of H_2O (3 mL). The reaction mixture was evaporated to dryness and the residue was subjected to column chromatography on silicagel (CH_2Cl_2/petroleum ether 80:20) to give maleimide **3e** (41 mg, 0.104 mmol, yield 54%) as a white solid. Mp: 98 °C. R_f (alumina, CH_2Cl_2/petroleum ether 80:20): 0.48. IR υ_{max}: 2933, 2853, 1701 (C=O), 1562, 1407, 1369. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.3–1.7 (m, 8H), 2.62 (s, 6H), 3.50 (t, 2H, J=6.5), 3.51 (t, 2H, J=7.4), 4.55 (s, 2H), 6.66 (s, 2H), 7.14 (d, 1H, J=7.5), 7.17 (s, 1H), 7.67 (t, 1H, J=7.8), 8.07 (s, 1H), 8.16 (d, 1H, J=7.8). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 24.7 (CH_3), 25.75 (CH_2), 26.6 (CH_2), 28.5 (CH_2), 29.6 (CH_2), 37.8 (CH_2), 70.9 (CH_2), 71.5 (CH_2), 116.6 (CH), 118.4 (CH), 121.2 (CH), 123.1 (CH), 134.1 (CH), 137.0 (CH), 148.9 (Cq), 155.9 (Cq), 156.0 (Cq), 157.9 (Cq), 158.2 (Cq), 170.9 (Cq). MS (ESI$^+$): m/z (%) 394.2 (100) $[M+H]^+$, 416.2 (9) $[M+Na]^+$. Anal. Calcd for $C_{23}H_{27}N_3O_3$: C, 70.21; H, 6.92; N, 10.68. Found: C, 70.47; H, 7.23; N, 10.32.

4.17. 4-(6-Azido-hexyloxymethyl)-6,6′-dimethyl-2,2′-bipyridine (3f)

To a stirred solution of bromide **3b** (90 mg, 0.239 mmol) in anhydrous DMF (1 mL) was added under Argon sodium azide (90 mg, 1.38 mmol). The reaction mixture was stirred at rt for 14 h and a saturated NH_4Cl solution (5 mL) was added. The mixture was extracted with CH_2Cl_2 and the organic layer was dried over $MgSO_4$. Evaporation of the solvent afforded the title compound **3f** (80 mg, 0.236 mmol, yield 99%) as a yellow oil. R_f (alumina, CH_2Cl_2/petroleum ether 60:40): 0.48. IR υ_{max}: 2936, 2860, 2095 (N_3), 1586, 1570, 1418, 1260. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.40–1.43 (m, 4H), 1.62–1.67 (m, 4H), 2.63 (s, 6H), 3.26 (t, 2H, J=6.9), 3.52 (t, 2H, J=6.5), 4.56 (s, 2H), 7.15 (d, 1H, J=7.8), 7.17 (s, 1H), 7.68 (t, 1H, J=7.8), 8.10 (s, 1H), 8.17 (d, 1H, J=7.8). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 24.5 (CH_3), 25.6 (CH_2), 26.4 (CH_2), 28.7 (CH_2), 29.4 (CH_2), 51.2 (CH_2), 70.7 (CH_2), 71.4 (CH_2), 116.4 (CH), 118.2 (CH), 121.0 (CH), 123.0 (CH), 136.9 (CH), 148.7 (Cq), 155.7 (Cq), 155.9 (Cq), 157.7 (Cq), 158.0 (Cq). MS (ESI$^+$): m/z (%) 340.3 (100) $[M+H]^+$, 362.4 (54) $[M+Na]^+$. Anal. Calcd for $C_{19}H_{25}N_5O$: C, 67.23; H, 7.42; N, 20.63. Found: C, 66.79; H, 7.74; N, 20.78.

4.18. 6,6′-Dimethyl-4-(oct-7-ynyloxymethyl)-2,2′-bipyridine (3g)

4.18.1. 7-Octyn-1-ol

Under Argon, lithium (140 mg, 20 mmol) was added to dry 1,3-diaminopropane (10 mL), and the mixture was heated at 70 °C for 3 h. At that time, the starting dark blue color was faded and a green suspension of the lithium salt was obtained. The reaction was cooled to rt and potassium *tert*-butoxide (1.34 g, 12 mmol) was added, affording a yellow solution. After stirring for 30 min at rt, 2-octyn-1-ol (425 μL, 3 mmol) was added via a syringe. The orange-brown solution was stirred at rt for a further 2 h and the resulting beige mixture was poured into water (50 mL). The mixture was extracted with CH_2Cl_2 (3×20 mL) and the combined organic extracts were washed successively with 10% HCl and saturated NaCl solution. After drying over $MgSO_4$, the organic layer was evaporated under reduced pressure to give the title compound (380 mg, 3 mmol, yield 100%) as an oil. The title compound was used without purification for the next reaction step. R_f (silica gel, CH_2Cl_2): 0.17. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.3–1.6 (m, 8H), 1.90 (t, 1H, J=2.6), 2.20 (td, 2H, J=6.8, 2.6), 3.68 (t, 2H, J=6.5). ^{1}H NMR data are in good agreement with the literature.[64]

4.18.2. 8-Bromo-1-octyne

To a stirred solution of 7-octyn-1-ol (1 g, 7.93 mmol) and PPh_3 (2.70 g, 10.3 mmol) in CH_2Cl_2 (10 mL), at 0 °C under Argon, was slowly added solid *N*-bromosuccinimide (1.83 g, 10.3 mmol), at such a rate that the temperature did not rise above 10 °C. The resulting orange mixture was warmed up to rt, and stirring was continued for 2 h. The solvent was removed under reduced pressure and the solid orange residue was extracted with petroleum ether. The extract was filtered, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography over silica gel (petroleum ether) to give the title compound (0.9 g, 4.76 mmol, yield 60%) as a colorless oil. R_f (silica gel, petroleum ether): 0.22. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.3–1.6 (m, 6H), 1.90 (m, 2H), 2.0 (t, 1H, J=2.6), 2.20 (td, 2H, J=6.8, 2.6), 3.40 (t, 2H, J=6.8). ^{1}H NMR data are in good agreement with the literature.[64,65]

4.18.3. Compound 3g

To a stirred solution of alcohol **2b** (100 mg, 0.467 mmol) in anhydrous THF (1.5 mL) was added under Argon sodium hydride (22.4 mg, 0.933 mmol). The resulting mixture was heated to reflux for 1 h, followed by addition of 8-bromo-1-octyne (265 mg, 1.4 mmol). The resulting solution was heated to reflux under Argon for 16 h. After cooling to rt, the reaction mixture was evaporated to dryness and the residue was subjected to column chromatography over alumina (petroleum ether then petroleum ether/CH_2Cl_2 60:40) to give acetylenic compound **3g** (54 mg, 0.168 mmol, yield 36%) as a waxy white solid. R_f (alumina, petroleum ether/CH_2Cl_2 60:40): 0.38. IR υ_{max}: 3300 (CH), 2936, 2859, 2110 (C≡C), 1586, 1570, 1419, 1117. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.39–1.69 (m, 8H), 1.93 (t, 1H, J=2.6), 2.18 (td, 2H, J=6.8, 2.6), 2.61 (s, 6H), 3.51 (t, 2H, J=6.5), 4.55 (s, 2H), 7.13 (d, 1H, J=7.5), 7.17 (s, 1H), 7.66 (t, 1H, J=7.7), 8.09 (s, 1H), 8.16 (d, 1H, J=7.8). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 18.3 (CH_2), 24.7 (CH_3), 25.7 (CH_2), 28.4 (CH_2), 28.5 (CH_2), 29.6 (CH_2), 68.2 (CH), 70.8 (CH_2), 71.5 (CH_2), 84.6 (Cq), 116.5 (CH), 118.3 (CH), 121.2 (CH), 123.1 (CH), 137.0 (CH), 148.8 (Cq), 155.9 (Cq), 156.0 (Cq), 157.8 (Cq), 158.2 (Cq). MS (ESI$^+$): m/z (%) 323.1 (100) $[M+H]^+$, 345.3 (23) $[M+Na]^+$.

4.19. 4-Carbomethoxy-6,6′-dimethyl-2,2′-bipyridine N_1,$N_{1'}$-dioxide (16)

To a stirred solution of *m*-CPBA (3.26 g, 18.89 mmol) in chloroform (27 mL) was added a solution of bipyridine **1** (1.20 g,

4.96 mmol) in chloroform (18 mL). The resulting solution was stirred at rt for 19 h, then neutralized with satd aq $NaHCO_3$, followed by extraction with CH_2Cl_2 (3×40 mL). The combined organic layers were dried (Na_2SO_4) and concentrated, to yield a yellow paste. The crude product was purified by column chromatography over silica gel (CH_2Cl_2/MeOH 97:3) to give dioxide **16** (1.29 g, 4.70 mmol, yield 95%) as a pale yellow solid. Mp: 222–224 °C. R_f (silica gel, CH_2Cl_2/MeOH 95:5): 0.10. IR υ_{max}: 3075, 1721 (C=O), 1426, 1329, 1261, 998. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.56 (s, 3H), 2.57 (s, 3H), 3.91 (s, 3H), 7.23 (t, 1H, J=7.8), 7.33 (dd, 1H, J=7.8, 2.1), 7.38 (dd, 1H, J=7.5, 2.1), 7.94 (d, 1H, J=2.7), 7.96 (d, 1H, J=2.4). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 17.77 (CH_3), 17.82 (CH_3), 52.6 (CH_3), 124.1 (CH), 124.5 (Cq), 125.3 (CH), 125.5 (CH), 126.6 (CH), 127.0 (CH), 142.9 (Cq), 143.8 (Cq), 149.7 (Cq), 149.8 (Cq), 164.1 (Cq). MS (DCI$^+$, NH_3): m/z (%) 275 (73) $[M+H]^+$, 259 (75) $[M-O+H]^+$, 243 (100) $[M-2O+H]^+$.

4.20. 4-Carbomethoxy-6,6′-bis(bromomethyl)-2,2′-bipyridine (4a)

Bipyridine dioxide **16** (758 mg, 2.76 mmol) was dissolved in acetic anhydride (10 mL), and the resulting solution was heated to 120 °C under Argon for 16 h with stirring. The reaction mixture was evaporated to dryness, followed by addition of a 33% solution of HBr in glacial acetic acid (19 mL). The resulting solution was heated to 70 °C under Argon for 7 h. The reaction mixture was cooled to rt and diluted with H_2O (190 mL), and the resulting solution was neutralized with satd aq $NaHCO_3$ and extracted with CH_2Cl_2 (6×50 mL). The combined organic layers were dried (Na_2SO_4), and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography over silica gel (CH_2Cl_2) to give dibromide **4a** (695 mg, 1.74 mmol, yield 63%) as a pale yellow solid. Mp: 127–128 °C. R_f (silica gel, CH_2Cl_2): 0.31. IR υ_{max}: 3077, 2949, 1730 (C=O), 1566, 1401, 1345, 1269, 1234, 1202, 771. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.01 (s, 3H), 4.65 (s, 2H), 4.67 (s, 2H), 7.51 (dd, 1H, J=7.8, 0.9), 7.84 (t, 1H, J=7.8), 8.01 (d, 1H, J=1.5), 8.40 (dd, 1H, J=7.8, 0.9), 8.89 (d, 1H, J=1.5). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 33.4 (CH_2), 33.9 (CH_2), 52.9 (CH_3), 119.9 (CH), 120.6 (CH), 122.8 (CH), 124.1 (CH), 138.1 (CH), 139.6 (Cq), 154.5 (Cq), 156.5 (Cq), 156.7 (Cq), 157.3 (Cq), 165.4 (Cq). MS (ESI$^+$): m/z (%) 401.3 (100) $[M+H]^+$, 423.0 (95) $[M+Na]^+$. Anal. Calcd for $C_{14}H_{12}N_2O_2Br_2$: C, 42.03; H, 3.02; N, 7.00. Found: C, 42.10; H, 3.15; N, 6.78.

4.21. 4-(*tert*-Butyldiphenylsilanyloxymethyl)-6,6′-dimethyl-2,2′-bipyridine (17)

To a stirred solution of alcohol **2b** (1.40 g, 6.53 mmol) in anhydrous DMF (65 mL) was added, at rt under Argon, imidazole (1.34 g, 19.7 mmol), followed by the dropwise addition of *tert*-butyldiphenylchlorosilane (2.7 mL, 10.5 mmol). The resulting solution was stirred at rt for 48 h. The reaction mixture was quenched with satd aq $NaHCO_3$ (60 mL), diluted with EtOAc (400 mL), sequentially washed with H_2O (2×200 mL) and brine (200 mL). The organic layer was dried (Na_2SO_4), and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography over alumina (Et_2O/petroleum ether 10:90), to give silyl ether **17** (2.77 g, 6.12 mmol, yield 94%) as a white solid. Mp: 89–91 °C. IR υ_{max}: 3070, 2958, 2930, 2857, 1585, 1570, 1427, 1418, 1113. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.18 (s, 9H), 2.63 (s, 3H), 2.67 (s, 3H), 4.86 (s, 2H), 7.15 (d, 1H, J=7.8), 7.28 (s, 1H), 7.38–7.46 (m, 6H), 7.69 (t, 1H, J=7.8), 7.72–7.75 (m, 4H), 8.15 (s, 1H), 8.21 (d, 1H, J=7.8). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 19.3 (Cq), 24.6 (CH_3), 24.8 (CH_3), 26.8 (CH_3), 64.4 (CH_2), 115.3 (CH), 118.1 (CH), 119.9 (CH), 122.9 (CH), 127.8 (CH), 129.8 (CH), 133.1 (Cq), 135.5 (CH), 136.9 (CH), 151.1 (Cq), 155.7 (Cq), 155.9 (Cq), 157.7 (Cq), 158.0 (Cq). MS (FAB$^+$, MNBA): m/z (%) 453 (100) $[M+H]^+$. Anal. Calcd for $C_{29}H_{32}N_2OSi$: C, 76.95; H, 7.13; N, 6.19. Found: C, 76.76; H, 7.21; N, 6.01.

4.22. 4-(*tert*-Butyldiphenylsilanyloxymethyl)-6,6′-dimethyl-2,2′-bipyridine N_1,$N_{1'}$-dioxide (18)

To a stirred solution of bipyridine **17** (1.00 g, 2.21 mmol) in $CHCl_3$ (75 mL) was added dropwise a solution of *m*-CPBA (1.50 g, 8.69 mmol) in $CHCl_3$ (55 mL), and the resulting solution was stirred at rt for 24 h. The reaction mixture was washed with satd aq $NaHCO_3$ (2×30 mL). The organic layer was dried (Na_2SO_4), and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography over silica gel (CH_2Cl_2/MeOH 95:5, then 80:20), to give dioxide **18** (844 mg, 1.74 mmol, yield 78%) as a tan solid. Mp: 67 °C. IR υ_{max}: 3071, 2931, 2858, 1471, 1446, 1428, 1370, 1253, 1113. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.10 (s, 9H), 2.57 (s, 6H), 4.71 (s, 2H), 7.21–7.45 (m, 11H), 7.66 (dd, 4H, J=7.5, 1.5). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 17.8 (CH_3), 18.0 (CH_3), 19.2 (Cq), 26.7 (CH_3), 63.5 (CH_2), 122.3 (CH), 123.8 (CH), 124.0 (CH), 125.1 (CH), 126.6 (CH), 129.9 (CH), 132.6 (Cq), 135.7 (CH), 138.1 (Cq), 142.9 (Cq), 143.7 (Cq), 149.0 (Cq), 149.5 (Cq). MS (FAB$^+$, MNBA): m/z (%) 485 (100) $[M+H]^+$, 507 (7) $[M+Na]^+$.

4.23. 4-(*tert*-Butyldiphenylsilanyloxymethyl)-6,6′-diacetoxymethyl-2,2′-bipyridine (19)

A solution of 450 mg (0.93 mmol) of dioxide **18** in acetic anhydride (7 mL) was heated to 100 °C with stirring for 24 h. The reaction mixture was concentrated, and the residue was purified by flash chromatography over alumina (Et_2O/petroleum ether 45:55), to give diacetate **19** (360 mg, 0.63 mmol, yield 68%) as a pale yellow solid. Mp 126–128 °C. IR υ_{max}: 3071, 2932, 2857, 1746 (C=O), 1588, 1427, 1224, 1113. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.18 (s, 9H), 2.18 (s, 3H), 2.20 (s, 3H), 4.90 (s, 2H), 5.29 (s, 2H), 5.35 (s, 2H), 7.36–7.50 (m, 8H), 7.73 (dd, 4H, J=7.5, 1.5), 7.83 (t, 1H, J=7.8), 8.33 (s, 1H), 8.38 (d, 1H, J=7.8). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 19.3 (Cq), 20.9 (CH_3), 26.5 (CH_3), 26.8 (CH_3), 64.3 (CH_2), 66.9 (CH_2), 67.0 (CH_2), 117.1 (CH), 118.2 (CH), 120.3 (CH), 121.5 (CH), 127.8 (CH), 129.9 (CH), 132.9 (Cq), 135.4 (CH), 137.5 (CH), 152.1 (Cq), 155.15 (Cq), 155.2 (Cq), 155.5 (Cq), 170.6 (Cq). MS (ESI$^+$): m/z (%) 569.7 (100) $[M+H]^+$, 591.3 (77) $[M+Na]^+$. Anal. Calcd for $C_{33}H_{36}N_2O_5Si$: C, 69.69; H, 6.38; N, 4.93. Found: C, 69.68; H, 6.56; N, 5.38.

4.24. 4-(*tert*-Butyldiphenylsilanyloxymethyl)-6,6′-bis(hydroxymethyl)-2,2′-bipyridine (20)

To a stirred suspension of diacetate **19** (360 mg, 0.63 mmol) in MeOH (6.5 mL) and H_2O (0.6 mL) was added solid potassium carbonate (350 mg, 2.5 mmol). The resulting mixture was vigorously stirred at rt for 3 h. The reaction mixture was diluted with CH_2Cl_2 (30 mL), washed sequentially with H_2O (2×10 mL) and brine (10 mL). The organic layer was dried (Na_2SO_4), and the solvent was removed under reduced pressure to give diol **20** (305 mg, 0.63 mmol, yield 100%) as a glassy pale yellow oil. IR υ_{max}: 3391, 3070, 2931, 2857, 1611, 1588, 1568, 1471, 1427, 1157, 1112. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.18 (s, 9H), 3.75–4.15 (s, br, 2H), 4.83 (s, 2H), 4.85 (s, 2H), 4.89 (s, 2H), 7.26 (d, 1H, J=7.5), 7.31 (s, 1H), 7.36–7.46 (m, 6H), 7.72 (dd, 4H, J=7.5, 1.5), 7.81 (t, 1H, J=7.8), 8.31 (s, 1H), 8.33 (d, 1H, J=7.8). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 19.3 (Cq), 26.8 (CH_3), 63.9 (CH_2), 64.3 (CH_2), 116.7 (CH), 117.5 (CH), 119.7 (CH), 120.6 (CH), 127.8 (CH), 129.9 (CH), 132.8 (Cq), 135.4 (CH), 137.6 (CH), 152.4 (Cq), 153.9 (Cq), 154.0 (Cq), 158.3 (Cq), 158.4 (Cq). MS (ESI$^+$): m/z (%) 485.3 (100) $[M+H]^+$, 507.3 (12) $[M+Na]^+$.

4.25. 4-(*tert*-Butyldiphenylsilanyloxymethyl)-6,6′-bis(chloromethyl)-2,2′-bipyridine (4b)

Diol **20** (160 mg, 0.33 mmol) was dissolved in thionyl chloride (3 mL) and stirred at rt for 1.5 h. The solution was evaporated in

vacuo and the residue was dissolved in CH_2Cl_2 and washed with a satd aq $NaHCO_3$ solution. The organic layer was dried ($MgSO_4$), evaporated in vacuo and the crude product was purified by column chromatography over alumina (petroleum ether/CH_2Cl_2 70:30) to give dichloro derivative **4b** (110 mg, 0.21 mmol, yield 64%) as a colorless oil. R_f (alumina, petroleum ether/CH_2Cl_2 70:30): 0.69. IR υ_{max}: 3062, 2954, 2930, 2857, 1606, 1587, 1566, 1462, 1427, 1413, 1262. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.16 (s, 9H), 4.72 (s, 2H), 4.76 (s, 2H), 4.88 (s, 2H), 7.36–7.46 (m, 6H), 7.50 (dd, 1H, J=7.7, 1.0), 7.58 (d, 1H, J=1.0), 7.69–7.72 (m, 4H), 7.84 (t, 1H, J=7.8), 8.36 (d, 1H, J=1.0), 8.39 (dd, 1H, J=7.8, 1.0). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 19.4 (Cq), 26.8 (CH_3), 46.8 (CH_2), 64.3 (CH_2), 117.6 (CH), 119.9 (CH), 120.6 (CH), 122.8 (CH), 127.9 (CH), 129.9 (CH), 132.9 (Cq), 135.5 (CH), 137.9 (CH), 152.7 (Cq), 155.1 (Cq), 155.2 (Cq), 156.0 (Cq), 156.1 (Cq). MS (ESI$^+$): m/z (%) 521.1 (9) [M+H]$^+$, 523.1 (7) [M+H]$^+$, 543.1 (100) [M+Na]$^+$, 545.1 (67) [M+Na]$^+$.

4.26. Tetra (*tert*-butyl) 2,2′,2″,2‴-{[4-(methoxycarbonyl)-2,2′-bipyridine-6,6′-diyl]bis(methylenenitrilo)}-tetrakis (acetate) (5a)

To a stirred solution of dibromide **4a** (800 mg, 2.0 mmol) in anhydrous acetonitrile (160 mL) was added Na_2CO_3 (2.12 g, 20.0 mmol) and di-*tert*-butyl iminodiacetate (1.03 g, 4.2 mmol). The resulting mixture was stirred at reflux for 17 h, then filtered, and the filtrate was concentrated in vacuo. The residue was taken up in CH_2Cl_2 (100 mL) and washed with H_2O (75 mL). The aqueous layer was extracted with CH_2Cl_2 (50 mL), the combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silicagel (CH_2Cl_2/MeOH 100:0, then 98:02) to give compound **5a** (1.33 g, 1.82 mmol, yield 91%) as a pale yellow oil. R_f (Silica gel, CH_2Cl_2/MeOH 95:05): 0.29. IR υ_{max}: 2978, 2932, 1734 (C=O), 1567, 1368, 1256, 1220, 1144, 991, 773. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.46 (s, 36H), 3.51 (s, 4H), 3.54 (s, 4H), 3.96 (s, 3H), 4.13 (s, 2H), 4.18 (s, 2H), 7.69 (dd, 1H, J=7.7, 1.1), 7.78 (t, 1H, J=7.7), 8.11 (d, 1H, J=1.4), 8.31 (dd, 1H, J=7.7, 1.1), 8.81 (d, 1H, J=1.4). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 28.1 (CH_3), 52.4 (CH_3), 55.7 (CH_2), 55.8 (CH_2), 59.4 (CH_2), 59.9 (CH_2), 80.97 (Cq), 81.02 (Cq), 118.8 (CH), 119.5 (CH), 122.0 (CH), 123.4 (CH), 137.3 (CH), 139.0 (Cq), 154.5 (Cq), 156.6 (Cq), 159.0 (Cq), 159.8 (Cq), 166.0 (Cq), 170.4 (Cq), 170.5 (Cq). MS (FAB$^+$/MNBA): m/z (%) 729 (100) [M+H]$^+$, 751 (30) [M+Na]$^+$. Anal. Calcd for $C_{38}H_{56}N_4O_{10}$: C, 62.62; H, 7.74; N, 7.69. Found: C, 62.47; H, 7.65; N, 7.47.

4.27. 2,2′,2″,2‴-[(4-Carboxy-2,2′-bipyridine-6,6′-diyl)bis(methylenenitrilo)]-tetrakis (acetic acid) (5b)

The ester **5a** (77 mg, 0.106 mmol) in a 6 N HCL solution (5 mL) was stirred at 90 °C for 3 days. The solvent was removed under reduced pressure to give compound **5b** in the form of its hydrochloride salt as a white solid (64 mg, 0.106 mmol, yield 100%). IR υ_{max}: 3385, 3185, 3009, 1723 (C=O), 1591, 1572, 1510. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.67 (s, 8H), 4.20 (s, 2H), 4.23 (s, 2H), 7.66 (d, 1H, J=7.7), 7.99 (t, 1H, J=7.7), 8.06 (d, 1H, J=1.3), 8.31 (d, 1H, J=7.7), 8.69 (d, 1H, J=1.3). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 54.2 (CH_2), 54.3 (CH_2), 58.9 (CH_2), 59.2 (CH_2), 118.3 (CH), 119.6 (CH), 122.6 (CH), 124.1 (CH), 138.2 (CH), 140.1 (Cq), 153.7 (Cq), 155.4 (Cq), 157.3 (Cq), 158.8 (Cq), 166.2 (Cq), 171.3 (Cq), 171.5 (Cq). MS (ESI$^+$): m/z (%) 491.3 (100) [M+H]$^+$, 592.2 (95) [M+K]$^+$.

4.28. Labeling of BSA with 5b·Eu^{3+}

BSA was labeled with **5b**·Eu^{3+} by using succinimidyl monoester (NHS-**5b**·Eu^{3+}) of **5b**·Eu^{3+}. The preparation of **5b**·Eu^{3+} and the labeling procedure are described in the following.

4.28.1. Synthesis of 5b·Eu^{3+}

A solution of **5b** (2.95 mg, 6.0 μmol) with $EuCl_3 \cdot 6H_2O$ (1.1 equiv) in 2 mL of H_2O at pH 3 was stirred at room temperature for 24 h. The excess of Eu^{3+} was precipitated by adjusting the pH to 8 using NaOH solution. The solvent was removed, the resulting solid was dissolved in a minimum of methanol, and diethylether was added to precipitate the compound **5b**·Eu^{3+}. MS (ESI$^-$, H_2O): m/z (%) 637.37 (94) [M−H]$^-$, 639.37 (100) [M−H]$^-$, 661.38 (18) [M+Na−2H]$^-$, 661.38 (20) [M+Na−2H]$^-$. Luminescence (H_2O, λ_{exc}=317 nm): λ_{em} (relative intensity, corrected spectrum), 580 (2.0), 592 (31.6), 618 (100), 651 (5.6), 697 (74.2) nm. $\tau(H_2O)$=0.59 ms.

4.28.2. Labeling of BSA with NHS-5b·Eu^{3+}

5b·Eu^{3+} (1.38 mg, 2.2 μmol) was dissolved in 100 μL of anhydrous DMF. Stocks solutions of EDCI·HCl (44 μL, 2.2 μmol) and *N*-hydroxysuccinimide (11.4 μL, 2.2 μmol) in anhydrous DMF were added, and the solution was stirred at rt for 24 h. Bovine serum albumin (4.78 mg, 72 nmol) dissolved in 480 μL of phosphate buffer (50 mM, pH=8) was added to achieve a 30:1 label/protein reaction ratio, and the mixture was allowed to stir for 48 h at room temperature. The reaction mixture was concentrated to a low volume using a Microcon YM-10 centrifugal filter (Amicon, Millipore, Bedford, MA), and then 400 μL of H_2O was added and the solution was concentrated again. This operation was repeated once again to remove totally the unbound label. The extent of conjugation was determined by MALDI-TOF-MS. The matrix was prepared from a solution of sinapinic acid in acetonitrile. The sample and the matrix solution were mixed in equal amounts (1 μL) and added on the stainless steel probe. 1 μL of aqueous 0.1% TFA was added to each spot and the sample was allowed to dry at room temperature. The data were acquired in the linear mode. The comparison of the molecular mass of the Europium labeled BSA (67,418.2 Da) with that of the starting protein (66,401.1 Da) allowed establishing that the mean number of europium complexes labels per BSA molecule is 1.6. **5b**·Eu^{3+}-BSA: Luminescence (H_2O, λ_{exc}=317 nm): λ_{em} (relative intensity, corrected spectrum), 580 (6.0), 592 (29.8), 618 (100), 651 (5.5), 697 (65.9) nm. $\tau(H_2O)$=0.67 ms.

4.29. Tetra (*tert*-butyl) 2,2′,2″,2‴-{[4-(hydroxymethyl)-2,2′-bipyridine-6,6′-diyl]bis(methylenenitrilo)}-tetrakis (acetate) (5c)

To a solution of ester **5a** (729 mg, 1.0 mmol) in anhydrous methanol (50 mL) was added $NaBH_4$ as a solid (1.13 g, 30.0 mmol) in five portions (2 h) with stirring at rt under Argon. The resulting mixture was further stirred for 2 h at rt and the solvent removed under reduced pressure. A solution of satd aq NH_4Cl (15 mL) was added, and the resulting mixture was partitioned between CH_2Cl_2 (50 mL) and H_2O (50 mL). The aqueous layer was extracted with CH_2Cl_2 (30 mL), the combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure. The crude product was purified by column chromatography over alumina (CH_2Cl_2/MeOH 99:01) to give alcohol **5c** (652 mg, 0.93 mmol, yield 93%) as a colourless oil. R_f (Alumina, CH_2Cl_2/MeOH 99:01): 0.25. IR υ_{max}: 3357, 2980, 2933, 1734, 1639, 1588, 1457, 1394, 1369, 1249, 1155. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.49 (s, 36H), 3.54 (s, 4H), 3.55 (s, 4H), 4.14 (s, 4H), 4.73 (s, 2H), 7.65 (s, 1H), 7.66 (d, 1H, J=7.7), 7.79 (t, 1H, J=7.7), 8.33 (d, 1H, J=6.7), 8.34 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 28.1 (CH_3), 55.7 (CH_2), 59.6 (CH_2), 59.8 (CH_2), 63.9 (CH_2), 81.0 (Cq), 117.0 (CH), 119.6 (CH), 120.4 (CH), 123.0 (CH), 137.3 (CH), 151.6 (Cq), 155.3 (Cq), 155.6 (Cq), 158.5 (Cq), 158.7 (Cq),

170.5 (Cq). MS (CI/NH_3): *m/z* (%) 701.6 (55) $[M+H]^+$, 718.6 (100) $[M+NH_4]^+$.

4.30. Tetra (*tert*-butyl) 2,2′,2″,2‴-{[4-(prop-2-ynyloxymethyl)-2,2′-bipyridine-6,6′-diyl]bis(methylenenitrilo)}-tetrakis (acetate) (5d)

To a stirred solution of alcohol **5c** (100 mg, 0.14 mmol) in anhydrous DMF (2 mL) was added at rt under Argon sodium hydride (6.9 mg, 0.28 mmol) in one portion, followed by the addition of a solution of propargyl bromide in toluene (80 wt%, 50 μL, 0.45 mmol). The resulting mixture was stirred at rt for 48 h, then a solution of satd aq NaCl (5 mL) was added. The mixture was extracted with CH_2Cl_2 (3×5 mL), the combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure. The crude product was purified by column chromatography over alumina (CH_2Cl_2/MeOH 98:02) to give acetylenic compound **5d** (42 mg, 0.057 mmol, yield 41%) as a colourless oil. R_f (Alumina, CH_2Cl_2/MeOH 98:02): 0.29. IR υ_{max}: 3270, 2978, 2931, 2116, 1733, 1609, 1585, 1566, 1456, 1417, 1393, 1368, 1254, 1221, 1147. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.49 (s, 36H), 2.51 (t, 1H, *J*=2.4), 3.55 (s, 4H), 3.56 (s, 4H), 4.15 (s, 4H), 4.29 (d, 2H, *J*=2.4), 4.72 (s, 2H), 7.61 (s, 1H), 7.67 (d, 1H, *J*=7.7), 7.80 (t, 1H, *J*=7.7), 8.30 (s, 1H), 8.32 (d, 1H, *J*=7.7). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 28.2 (CH_3), 55.74 (CH_2), 55.76 (CH_2), 57.9 (CH_2), 59.6 (CH_2), 59.9 (CH_2), 70.3 (CH_2), 75.1 (Cq), 79.3 (CH), 81.0 (Cq), 117.9 (CH), 119.6 (CH), 121.4 (CH), 123.1 (CH), 137.4 (CH), 148.0 (Cq), 155.3 (Cq), 155.8 (Cq), 158.8 (Cq), 158.9 (Cq), 170.61(Cq), 170.65 (Cq). MS (ESI$^+$): *m/z* (%) 739.7 (94) $[M+H]^+$, 761.6 (100) $[M+Na]^+$.

4.31. NaBr complex of 6,6′-{*N*,*N*′*N*,*N*′-[Bis (2,2′-bipyridine-6,6′-dimethyl)]bis (aminomethyl)}-2,2′-bipyridine-4-carboxylic acid methyl ester (6a·Na^+)

To a stirred suspension of macrocyclic diamine **23** (120 mg, 0.3 mmol) and Na_2CO_3 (318 mg, 3 mmol) in anhydrous CH_3CN (150 mL), at reflux under Argon, was added, dropwise over 20 min, a solution of dibromide **4a** (120 mg, 0.3 mmol) in anhydrous CH_3CN (50 mL). The resulting mixture was stirred at reflux for 48 h. The reaction mixture was filtered and concentrated in vacuo, to yield a pale yellow solid. The crude product was purified by flash chromatography over silica gel (CH_2Cl_2/MeOH 93:7), to give sodium cryptate **6a**·Na^+ (109 mg, 0.14 mmol, yield 47%) as a yellow solid. Mp>250 °C (dec). IR υ_{max}: 3421, 2921, 1728 (C=O), 1590, 1577, 1433, 1267, 1171, 996. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.81 (br s, 10H), 3.91 (br s, 2H), 3.95 (s, 3H), 7.27–7.36 (m, 4H), 7.74–7.94 (m, 12H), 8.35 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 52.9 (CH_3), 59.4 (CH_2), 119.3 (CH), 120.2 (CH), 120.3 (CH), 120.4 (CH), 122.8 (CH), 124.0 (CH), 124.5 (CH), 138.10 (CH), 138.15 (CH), 138.2 (CH), 139.2 (Cq), 154.3 (Cq), 155.05 (Cq), 155.15 (Cq), 156.4 (Cq), 158.1 (Cq), 158.3 (Cq), 158.7 (Cq), 159.8 (Cq), 164.9 (Cq). MS (FAB$^+$, MNBA): *m/z* (%) 655 (100) $[M+Na]^+$, 671 (9) $[M+K]^+$. HRMS-FAB$^+$ calcd for $[M+Na]^+$ ($C_{38}H_{32}N_8O_2Na$) 655.25459, found 655.25382. Anal. Calcd for $C_{38}H_{32}N_8O_2NaBr\cdot 2H_2O$: C, 59.15; H, 4.70; N, 14.52. Found: C, 58.82; H, 4.89; N, 14.28.

4.32. General procedure for the preparation of lanthanide complexes of Cryptand 6a

To a stirred solution of the lanthanide salt $LnCl_3\cdot 6H_2O$ (1.1 equiv) in methanol (20 ml) was added sodium cryptate **6a**·Na^+ (46 mg, 0.06 mmol, 1 equiv) in chloroform (10 mL). After 24 h of reflux, the solvents were evaporated, and the residue dissolved in the minimum of methanol. Anhydre diethyl ether was added carefully until the apparition of a slight trouble. The mixture was cooled to 4 °C, and the resulting precipitate was isolated after centrifugation.

Complex **6a**·$EuCl_3$: MS (FAB$^+$, MNBA): *m/z* (%) 855 (50) $[M-Cl]^+$, 819 (100) $[M-Cl-HCl]^+$. Luminescence (H_2O λ_{exc}=306 nm): λ_{em} (relative intensity, corrected spectrum), 581 (3.3), 592 (42.0), 617 (81.8), 651 (6.8), 702 (100) nm.

Complex **6a**·$GdCl_3$: MS (FAB$^+$, MNBA): *m/z* (%) 860 (53) $[M-Cl]^+$, 824 (100) $[M-Cl-HCl]^+$.

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References and notes

1. Reedijk, J. In *Comprehensive Coordination Chemistry*; Wilkinson, G., Sir, Gillard, R. D., McCleverty, J. A., Eds.; Pergamon: Oxford, NY, 1987; Vol. 2, pp 73–98.
2. Chelucci, G.; Thummel, R. P. *Chem. Rev.* **2002**, *102*, 3129–3170.
3. Balzani, V.; Juris, A.; Venturi, M.; Campagna, S.; Serroni, S. *Chem. Rev.* **1996**, *96*, 759–833.
4. Sun, L.; Hammarström, L.; Åkermark, B.; Styring, S. *Chem. Soc. Rev.* **2001**, *30*, 36–49.
5. Keefe, M. H.; Benkstein, K. D.; Hupp, J. T. *Coord. Chem. Rev.* **2000**, *205*, 201–228.
6. Maury, O.; Le Bozec, H. *Acc. Chem. Res.* **2005**, *38*, 691–704.
7. Carlson, B.; Phelan, G. D.; Kaminsky, W.; Dalton, L.; Jiang, X.; Liu, S.; Jen, A. K.-Y. *J. Am. Chem. Soc.* **2002**, *124*, 14162–14172.
8. Schubert, U. S.; Eschbaumer, C. *Angew. Chem., Int. Ed.* **2002**, *41*, 2892–2926.
9. Hemmilä, I.; Laitala, V. *J. Fluoresc.* **2005**, *15*, 529–542.
10. Faulkner, S.; Pope, S. J. A.; Burton-Pye, B. P. *Appl. Spectrosc. Rev.* **2005**, *40*, 1–31.
11. Bünzli, J.-C. G. In *Metal Ions in Biological Systems*; Sigel, A., Sigel, H., Eds.; Marcel Dekker: New York, NY, 2004; Vol. 42, pp 39–75.
12. Brunet, E.; Juanes, O.; Rodriguez-Ubis, J.-C. *Curr. Chem. Biol.* **2007**, *1*, 11–39.
13. Nasso, I.; Galaup, C.; Havas, F.; Tisnès, P.; Picard, C.; Laurent, S.; Vander Elst, L.; Muller, R. N. *Inorg. Chem.* **2005**, *44*, 8293–8305.
14. Faulkner, S.; Beeby, A.; Carrié, M.-C.; Dadabhoy, A.; Kenwright, A. M.; Sammes, P. G. *Inorg. Chem. Commun.* **2001**, *4*, 187–190.
15. Sabbatini, N.; Guardigli, M.; Lehn, J.-M. *Coord. Chem. Rev.* **1993**, *123*, 201–228.
16. Alpha, B.; Ballardini, R.; Balzani, V.; Lehn, J.-M.; Perathoner, S.; Sabbatini, N. *Photochem. Photobiol.* **1990**, *52*, 299–306.
17. Bazin, H.; Trinquet, E.; Mathis, G. *Rev. Mol. Biotechnol.* **2002**, *82*, 233–250.
18. Arano, Y.; Uezono, T.; Akizawa, H.; Ono, M.; Wakisaka, K.; Nakayama, M.; Sakahara, H.; Konishi, J.; Yokoyama, A. *J. Med. Chem.* **1996**, *39*, 3451–3460.
19. Maisano, F.; Gozzini, L.; De Haen, C. *Bioconjugate Chem.* **1992**, *3*, 212–217.
20. Reilly, R.; Lee, N.; Houle, S.; Law, J.; Marks, A. *Appl. Radiat. Isot.* **1992**, *43*, 961–967.
21. Mukkala, V. M.; Sund, C.; Kwiatkowski, M. U.S. Patent 5,216,134, 1993; *Chem. Abstr.* **1993**, *119*, 244992.
22. Newkome, G. R.; Patri, A. K.; Holder, E.; Schubert, U. S. *Eur. J. Org. Chem.* **2004**, 235–254.
23. Nasso, I.; Bedel, S.; Galaup, C.; Picard, C. *Eur. J. Inorg. Chem.* **2008**, 2064–2074 and references cited therein.
24. Mathieu, J.; Marsura, A. *Synth. Commun.* **2003**, *33*, 409–414.
25. Bedel, S.; Ulrich, G.; Picard, C.; Tisnès, P. *Synthesis* **2002**, 1564–1570.
26. Havas, F.; Danel, M.; Galaup, C.; Tisnes, P.; Picard, C. *Tetrahedron Lett.* **2007**, *48*, 999–1002.
27. Regnouf de Vains, J.-B.; Papet, A. L.; Marsura, A. *J. Heterocycl. Chem.* **1994**, *31*, 1069–1077.
28. Kröhnke, F. *Synthesis* **1976**, 1–24.
29. Panetta, C. A.; Kumpaty, H. J.; Heimer, N. E.; Leavy, M. C.; Hussey, C. L. *J. Org. Chem.* **1999**, *64*, 1015–1021.
30. Heller, M.; Schubert, U. S. *J. Org. Chem.* **2002**, *67*, 8269–8272.
31. Fang, Y.-Q.; Hanan, G. S. *Synlett* **2003**, 852–854.
32. Hapke, M.; Brandt, L.; Lützen, A. *Chem. Soc. Rev.* **2008**, *37*, 2782–2797.
33. Adams, R.; Miyano, S. *J. Am. Chem. Soc.* **1954**, *76*, 3168–3171.
34. Schubert, U. S.; Eschbaumer, C.; Heller, M. *Org. Lett.* **2000**, *2*, 3373–3376.
35. Marvel, C. S.; Dreger, E. E. In *Organic Syntheses*; Blatt, A. H., Ed.; Wiley: New York, NY, 1941; Collect. Vol. 1, pp 238–240.
36. Libermann, D.; Rist, N.; Grumbach, F.; Cals, S.; Moyeux, M.; Rouaix, A. *Bull. Soc. Chim. Fr.* **1958**, 687–694.
37. Cuiban, F.; Cilianu-Bibian, S.; Zaharescu, L. Fr Patent 1,362,967, 1964.
38. Hodgson, P. B.; Salingue, F. H. *Tetrahedron Lett.* **2004**, *45*, 685–687.
39. Jones, N. A.; Antoon, J. W.; Bowie, A. L., Jr.; Borak, J. B.; Stevens, E. P. *J. Heterocycl. Chem.* **2007**, *44*, 363–367.
40. Pierrat, P.; Gros, P.; Fort, Y. *Org. Lett.* **2005**, *7*, 697–700.
41. Hanan, G. S.; Schubert, U. S.; Volkmer, D.; Rivière, E.; Lehn, J.-M.; Kyritsakas, N.; Fischer, J. *Can. J. Chem.* **1997**, *75*, 169–182.
42. Fallahpour, R. A. *Synthesis* **2000**, 1138–1142.
43. Amat, M.; Hadida, S.; Pshenichnyi, G.; Bosch, J. *J. Org. Chem.* **1997**, *62*, 3158–3175.

44. Fürstner, A.; Leitner, A.; Méndez, M.; Krause, H. *J. Am. Chem. Soc.* **2002**, *124*, 13856–13863.
45. Lehn, J.-M.; Mathis, G.; Alpha, B.; Deschenaux, R.; Jolu, E. Eur. Patent 321,353, 1989; *Chem. Abstr.* **1990**, *112*, 179041.
46. Brinkley, M. *Bioconjugate Chem.* **1992**, *3*, 2–13.
47. Mehta, N. B.; Phillips, A. P.; Lui, F. F.; Brooks, R. E. *J. Org. Chem.* **1960**, *25*, 1012–1015.
48. Walker, M. A. *Tetrahedron Lett.* **1994**, *35*, 665–668.
49. Kolb, H. C.; Sharpless, K. B. *Drug Discovery Today* **2003**, *8*, 1128–1137.
50. Abrams, S. R. *Can. J. Chem.* **1984**, *62*, 1333–1334.
51. Uenishi, J.; Tanaka, T.; Nishiwaki, K.; Wakabayashi, S.; Oae, S.; Tsukube, H. *J. Org. Chem.* **1993**, *58*, 4382–4388.
52. Liu, P.; Chen, Y.; Deng, J.; Tu, Y. *Synthesis* **2001**, 2078–2080.
53. Rodriguez-Ubis, J.-C.; Alpha, B.; Plancherel, D.; Lehn, J.-M. *Helv. Chim. Acta* **1984**, *67*, 2264–2269.
54. Horrocks, W. D. W., Jr.; Sudnick, D. R. *Acc. Chem. Res.* **1981**, *14*, 384–392.
55. Supkowski, R. M.; Horrocks, W. D. W., Jr. *Inorg. Chim. Acta* **2002**, *340*, 44–48.
56. Longo, R.; Gonçalves e Silva, F. R.; Malta, O. L. *Chem. Phys. Lett.* **2000**, *328*, 67–74.
57. Mathis, G.; Dumont, C.; Jolu, E. J. -P. WO Patent 92:01224, 1990; *Chem. Abstr.* **1992**, *116*, 147509.
58. Stein, G.; Würzberg, E. *J. Chem. Phys.* **1975**, *62*, 208–213.
59. Guillaumont, D.; Bazin, H.; Benech, J.-M.; Boyer, M.; Mathis, G. *ChemPhysChem* **2007**, *8*, 480–488.
60. Haas, Y.; Stein, G. *J. Phys. Chem.* **1971**, *75*, 3668–3677.
61. Nakamaru, K. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 2697–2705.
62. Galaup, C.; Carrié, M.-C.; Tisnès, P.; Picard, C. *Eur. J. Org. Chem.* **2001**, 2165–2175.
63. Henecka, H. *Chem. Ber.* **1949**, *82*, 36–41.
64. Patwardhan, A. P.; Thompson, D. H. *Langmuir* **2000**, *16*, 10340–10350.
65. Bauer, E. B.; Hampel, F.; Gladysz, J. A. *Adv. Synth. Catal.* **2004**, *346*, 812–822.