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Design and synthesis of europium luminescent bio-probes featuring sulfobetaine moieties

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ABSTRACT

Herein we report the straightforward preparation of chromophore-functionalized TACN ligand via Cu-free cross-coupling reactions using a common halogenated platform. This versatile methodology allows the preparation of original macrocyclic ligand featuring both optimized antenna for the sensitization of europium luminescence and sulfobetaine zwitterionic groups to ensure water solubility of the complex. In addition preliminary two-photon excited microscopy imaging experiments of fixed cells reveal that sulfobetaine groups are able to limit undesirable non specific interactions with biological surrounding.

The peculiar photophysical properties of f-block elements make them attractive candidates for the design of lanthanide luminescence bioprobes (LLB) for biological imaging or sensing applications.[1] An optimized LLB must fulfill a set of requirements namely (i) solubility and stability in water, and (ii) optimized chromophore antenna (Chrom) to ensure efficient sensitization. In this context, triazacyclononane macrocycle has become a platform of choice for the design of LLB for the following reasons: (i) its tris N-alkylation by pyridine carboxylate or phosphinate leads to the formation of a nonadentate ligand which can saturate the coordination sphere of lanthanide ion and form a stable complex in aqueous solutions.[2] (ii) In addition, following pioneering works of Takalo,[3] functionalization of the *para* position of the pyridine by chromophore antenna (Fig. 1, Chrom = aromatics, *p*-substituted aryl-ethynyl) enhances the absorption in the visible, while making it possible to tune its position. Recently, this strategy led to the preparation of Yb-bioprobes for thick tissue biphotonic microscopy imaging[4] and Eu-bioprobes with optimized brightness for one photon imaging[5] and sensing applications.[6] In each case, the ligand's synthesis proceeds according to the same convergent procedure (Fig 1A), involving the preparation of a Chrom-substituted picolinic arm followed by alkylation of the TACN moieties. This last step requires the use of excess Chrom-picolinic arm, which multistep synthesis requires lots of efforts, and which is hard to recover during purification.

In this Letter we describe an alternative versatile synthesis of various chromophore-functionalized triazacyclonane ligand $L^{n}_{(COOMe)}$, ($n = \mathbf{1}$–$\mathbf{3}$) using a divergent approach (Fig. 1B) involving the key synthon $\mathbf{L^{I3}}_{(COOMe)}$. The versatile character of the new synthetic methodology is first illustrated by the preparation of $\mathbf{L^{1,2}}_{(COOMe)}$ featuring methoxy donor groups, via Suzuki–Miyaura and copper-free Sonogashira cross-coupling reaction. In a second time, this procedure is applied for the design of the advanced ligand $\mathbf{L^{3}}_{(COOMe)}$ containing sulfobetaine end-groups. This zwitterionic fragment has been widely used for the hydrosolubilization of quantum dots or magnetic nanoparticles for bio-imaging applications[7] and it has been reported that the covering of the surface nanoparticles by sulfobetaine moieties presents the additional advantage of limiting the non specific interactions with the lipophilic biological surrounding compared to other neutral (poly-ethylene glycol) or charge (sulfonate, carboxylate, or ammonium) hydrosolubilizing functions.[7,8] This issue is crucial for any practical biological applications (imaging, sensing, and bioconjugation...) but has rarely been addressed for molecular probes. In this context, sulfobetaine groups are only present as polar fragments in the now commercially available amphiphilic membrane chromophore di-4-ANEPPS,[9] and have been recently introduced as hydrosolubilizing moieties in functional BODIPY dyes by Ziessel, Ulrich and co-workers.[10] To the best of our knowledge, this zwitterionic function has never been used in combination with lan-

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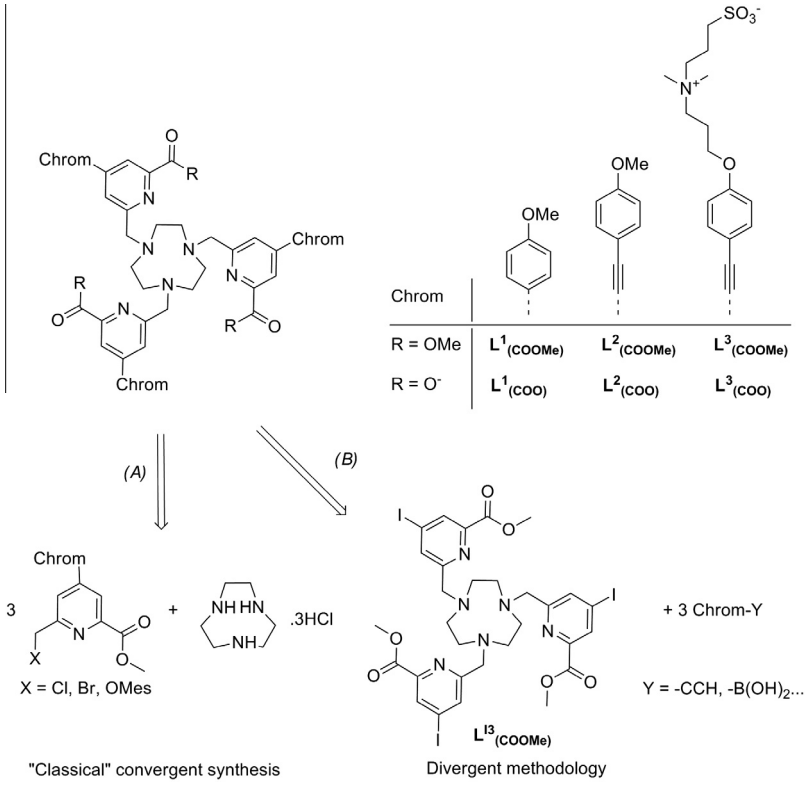


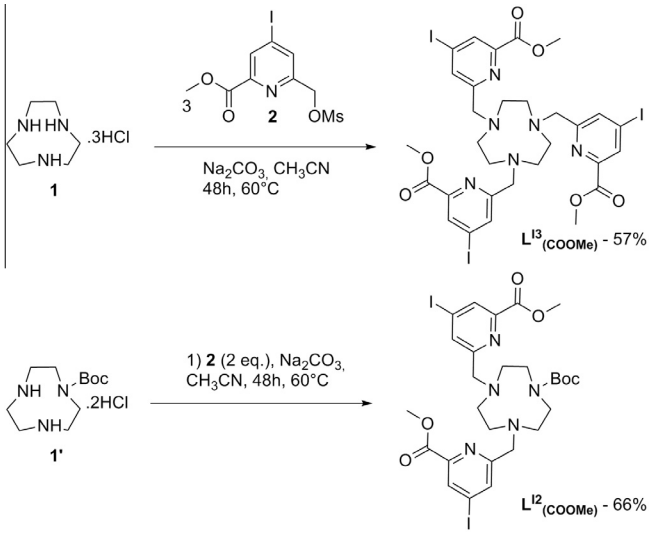
Figure 1. Retro-synthethic scheme for the preparation of target chromophore-TACN ligands.

thanide complexes. Here we described the design of [Eu·$\mathbf{L^3_{(COO)}}$], a LLB featuring sulfobetaine moieties enabling both hydrosolubilization and limitation of undesirable non-specific interactions with bio-molecules or lipophilic part of cells. The photophysic properties of the complex and preliminary two-photon bio-imaging experiments[11] are reported.

The 'classical' convergent synthesis illustrated in Figure 1, involving the alkylation of the TACN macrocycle in the last step, is not compatible with the presence of the dimethylamino peripheral moieties precursor of the sulfobetaine. Therefore an alternative synthetic pathway has been envisaged to tackle this issue, using the $\mathbf{L^{I3}_{(COOMe)}}$ ligand as a key functional platform. Moreover, this intermediate is an excellent starting point for a divergent and versatile one step preparation of various chromophore-ligands combinations via cross-coupling reaction with different Chrom-Y (Y is cross-coupling compatible function, typically terminal alkyne, boronic or stannane groups). This new synthetic pathway allows performing a systematic study of the influence of the antenna on the luminescence of the related Ln(III) complexes.

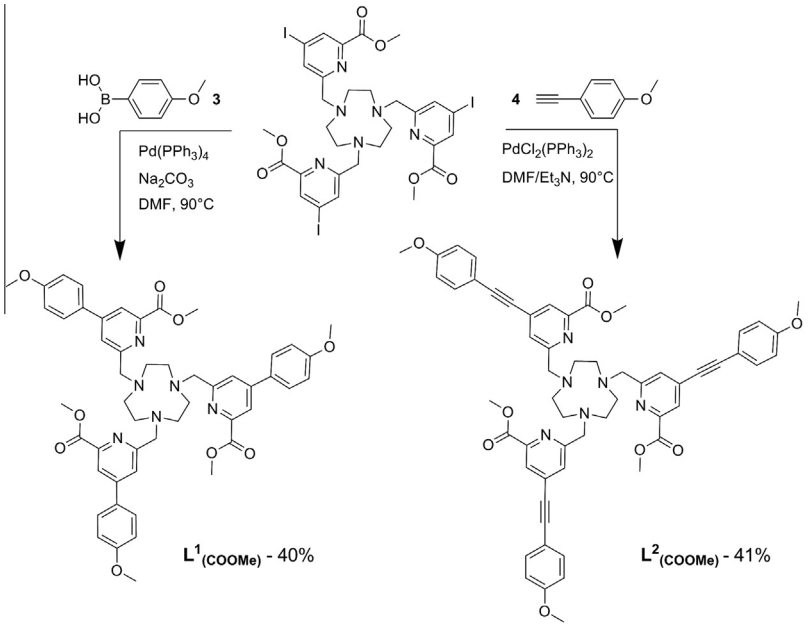
This $\mathbf{L^{I3}_{(COOMe)}}$ scaffold was obtained via a classical synthetic strategy, involving the alkylation of triazacyclononane trihydrochloride **1** by the mesylated derivative **2** in dry acetonitrile in the presence of sodium carbonate (Scheme 1). The synthesis was optimized and scaled-up to ca. 1.5 g (57%) after purification by simple precipitation in ethyl acetate.[12] This procedure was further extended to the preparation of the related dissymmetric $\mathbf{L^{I2}_{(COOMe)}}$ platform starting from the mono-boc protected TACN macrocycle, 1′.[5b,6a]

The $\mathbf{L^{I3}_{(COOMe)}}$ platform was first engaged in model cross coupling reactions to check its reactivity. Two kinds of cross-coupling reactions were envisioned, namely a Suzuki–Miyaura and a Sonogashira coupling to introduce the chromophore onto the triiodo ligand (Scheme 3). The Suzuki–Miyaura coupling with the commercially available anisole boronic acid (**3**) under classical conditions ($Pd(PPh_3)_4$, Na_2CO_3, DMF, 90 °C) led to the formation



Scheme 1. Synthesis of the iodo containing platform.

of the expected $\mathbf{L^1_{(COOMe)}}$ ligand in 40% yield after column chromatography.[13] On the other hand, Sonogashira coupling with anisole acetylene (**4**) under classical conditions ($PdCl_2(PPh_3)_2$, CuI, THF–NEt_3) was not successful. However, alternative conditions with $PdCl_2(PPh_3)_2$ as the catalyst in the absence of Cu(I) gave the expected $\mathbf{L^2_{(COOMe)}}$ ligand in 40% yield (Scheme 3). This result can be explained by the parasitic complexation of Cu(I) in the macrocyclic precursor. The apparently modest 40% yield is in fact the average of three coupling reactions in each picolinic moiety of the ligand and the effective yield of each coupling is around 75% which is classical for this type of ligands.[14] After optimization of the reaction conditions, we applied this synthetic procedure to

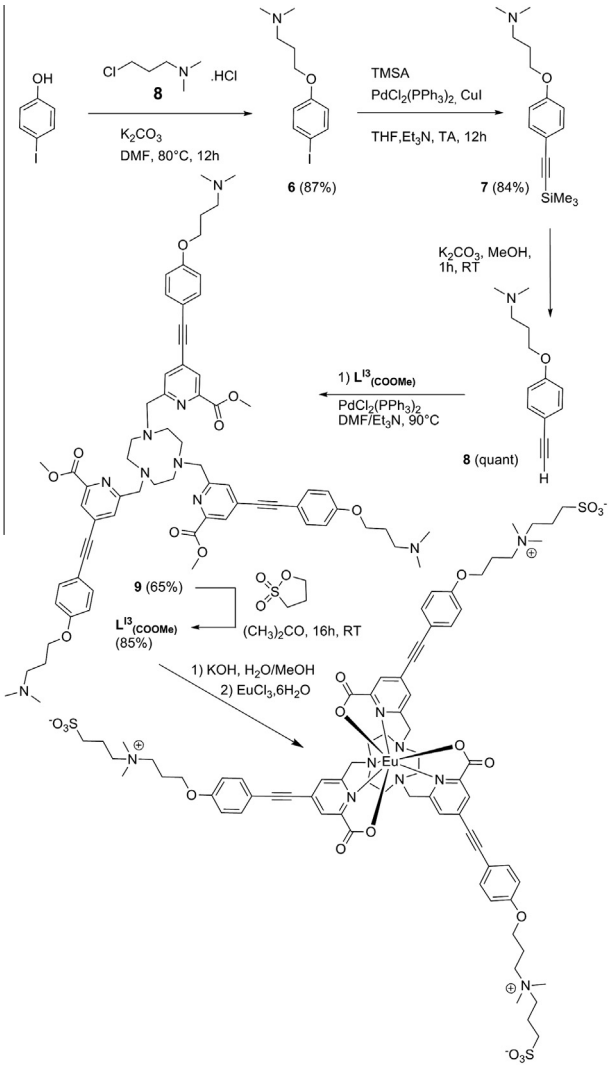


Scheme 2. Preparation of ligand $\mathbf{L^{1,2}_{(COOMe)}}$ using the synthetic pathway *B* via copper free Pd-catalyzed cross coupling reactions.

the introduction of different antenna containing various hydrosolubilizing groups like α-D-galactose derivative or sulfobetaine ones. In the following, we will only describe the synthesis of ligand $\mathbf{L^3_{(COOMe)}}$ featuring zwitterionic sulfobetaine substituent and that of the related [Eu·$\mathbf{L^3_{(COO)}}$] complex (Scheme 3).

The Chrom antenna **8** was first synthesized. First the terminal dimethylamino function was introduced by alkylation of 4-iodophenol by 1-dimethylamino-3-chloropropane. The resulting compound **6** was involved in a Sonogahira cross-coupling under classical conditions with trimethylsilylacetylene giving **7** in 84% yield after column chromatography (SiO_2, eluent CH_2Cl_2/MeOH gradient). Finally, the TMS group was removed to give **8** that was directly involved in the next step without purification. The Sonogashira coupling between $\mathbf{L^{I3}_{(COOMe)}}$ and **8** (4 equiv) was carried out using the copper free procedure described above and led to the formation of **9** in 65% yield after purification by three successive precipitations (AcOEt/C_5H_{12}). The final step was the quaternization of the dimethylamine moieties by opening of the 1,3-propanesultone. It is important to note that **9** presents *a priori* three sites for this quaternization reaction namely, the dimethylamino peripheral moieties, the tertiary amine of the TACN macrocycle, and the imino moieties of the picolinic ring. At room temperature, the opening of the 1,3-propanesultone occurs only on the less sterically hindered site, the dialkylamino one. Consequently the desired $\mathbf{L^3_{(COOMe)}}$ ligand was obtained and isolated in 85% yield after washing and trituration in dichloromethane. The final ligand and all intermediates were characterized by NMR and high-resolution mass spectrometry.[15] Complex [Eu·$\mathbf{L^3_{(COO)}}$] was prepared after in situ hydrolysis of the ester moieties and further addition of europium salt using reported procedure and was purified by dialysis in water.[4,5] Its structure is supported by mass spectrometry.[16]

This complex is perfectly soluble in water allowing to measure its spectroscopic properties. It presents a broad absorption spectrum centered at 337 nm ($\varepsilon = 32000\ M^{-1}\ cm^{-1}$) characteristic for this type of antenna.[5,17] Excitation in this transition resulted in the expected threefold symmetric Eu(III) luminescence profile (Fig. 2). The quantum yield (18%) and lifetime (0.8 ms) are in the same range although slightly lower than the corresponding polyethylene glycol substituted analogous (25%, 1.06 ms)[5] indicating



Scheme 3. Synthesis of $\mathbf{L^{I3}_{(COOMe)}}$.

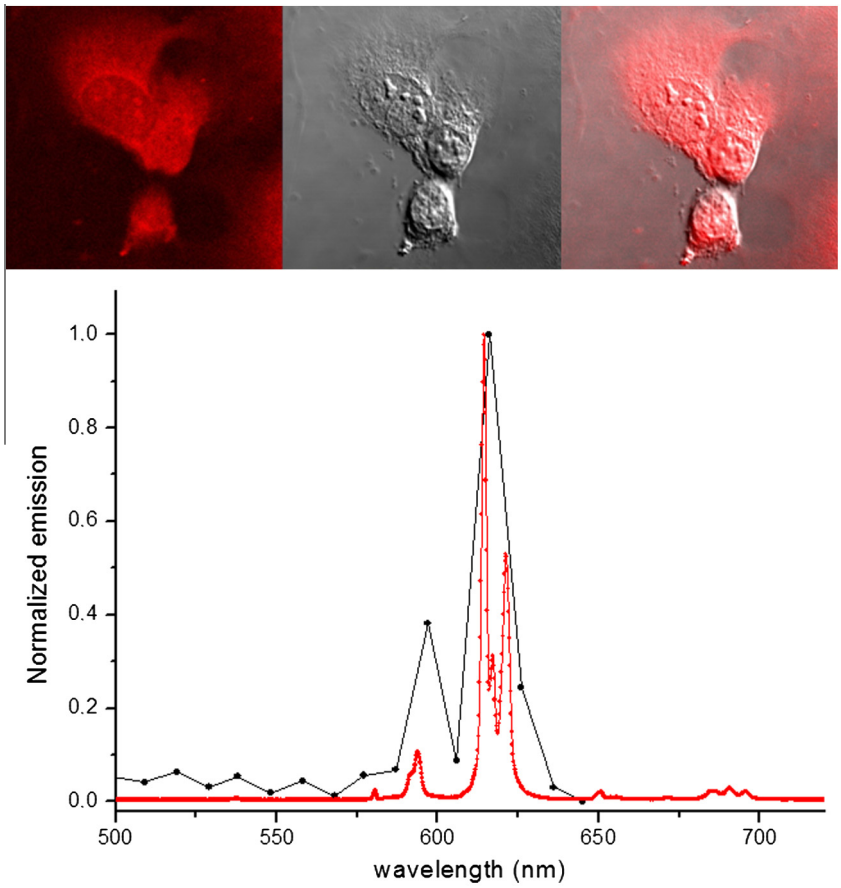


Figure 2. Comparison of the luminescence spectrum obtained in solution using a Jobin-Yvon Fluorolog apparatus (in red) and the two-photon excited luminescence spectrum obtained by the microscope using the spectral scanning mode. Inset from right to left: biphotonic image, phase contrast image and merged image of fixed human epithelial bladder cancer cell line (ATCC No HTB-4) cells stained by [Eu·$\mathbf{L^3_{(COO)}}$] using a microscope LSM-DuoScan NLO (Carl Zeiss) equipped with a Ti:Sa fs tunable laser (Chameleon, Ultra II, Coherent) (excitation 700 nm).

that modification of the peripheral solubilizing moieties does not significantly affect the complex photophysical properties. Fixed T24 cancer cells were stained with a solution of [Eu·$\mathbf{L^3_{(COO)}}$] ($c \sim 10^{-5}$ mol L^{-1}) and two-photon cell imaging experiments were successfully performed (λ^2 = 700 nm, Fig. 2).[18] The spectral analysis of the red glowing pixels corresponds well with the [Eu·$\mathbf{L^3_{(COO)}}$] luminescence spectra recorded independently, clearly confirming that the LLB is not altered in the cell. The localization of the sulfobetaine containing complex in the fixed cell seems to be identical to what had been previously observed with PEG or methyl substituted complexes.[4,5,19] However, the images look more diffuse, due to the ubiquitous presence of [Eu·$\mathbf{L^3_{(COO)}}$] in the endoplasmic reticulum without any specific localization. This behavior was confirmed by the complete disappearance of the complex, when the cells are rinsed with the medium. These observations are in marked contrast with PEG or methyl substituted and indicate that [Eu·$\mathbf{L^3_{(COO)}}$] does not accumulate in the lipophilic part of the cell. The non-specific interactions responsible for this complex accumulation are less prone to occur with [Eu·$\mathbf{L^3_{(COO)}}$] and this behavior can be ascribed to the presence of the zwitterionic sulfobetaine moieties (Scheme 2).

In conclusion, this article describes a new versatile synthesis of chromophore functionalized triazacyclononane macrocycles using a common halogenated platform, $\mathbf{L^{I3}_{(COOMe)}}$ by Suzuki–Miyaura or copper-free Sonogashira reactions. This new divergent methodology should be compatible with any type of cross-coupling, and should enable one to readily screen various chromophore antennae in order to optimize the sensitized lanthanide emission. Furthermore the synthesis of the di-halogenated $\mathbf{L^{I2}_{(COOMe)}}$ platform opens the way to the design of di-functionalized, or dissymmetric tri-functionalized triazacyclononane ligands for sensing applications[6] or bio-conjugation.[5] This synthetic methodology was successfully applied to the preparation of the ligand $\mathbf{L^3_{(COOMe)}}$ containing hydrosolubilizing zwitterionic sulfobetaine moities. The related europium(III) complex exhibits good emission properties in water and was involved in fixed cell imaging experiments using biphotonic microscopy. The staining was reversible upon rinsing indicating that the presence of sulfobetaine moieties limits strongly the non specific interactions with biological surrounding responsible for the accumulation of lipophilic organic dyes or complexes in membranes or organelles. The limitation of these non specific interactions is also a crucial issue for bioconjugation of emissive dyes or complexes with proteins or other biomacromolecules and the use of sulfobetaine moieties in this context is under study in our laboratory.[20]

Acknowledgments

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12. For smaller quantities column chromatography purification is preferable (Al_2O_3, act.III, eluant AcOEt). Selected spectroscopic data: $\mathbf{L^{I3}_{(COOMe)}}$. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.35 (d, J = 1.4 Hz, 3H), 8.21 (d, J = 1.4 Hz, 3H), 3.97 (s, 9H), 3.93(s, 6H), 2.90 (s, 12H). ^{13}C NMR (125.76 MHz, $CDCl_3$) δ: 164.7, 162.0, 147.7, 135.7, 132.9, 106.7, 64.0, 56.1, 53.3. HRMS ESI$^+$: calcd for $C_{30}H_{34}I_3N_6O_5$: 954.9627; found: $[MH]^+$ m/z = 954.9668.
13. *Selected spectroscopic data:* $\mathbf{L^1_{(COOMe)}}$. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.19 (s, 3H), 8.02 (s, 3H), 7.58 (d, 6H, J = 8.8 Hz), 6.94 (d, 6H, J = 8.9 Hz), 4.02 (s, 6H), 3.99 (s, 9H), 3.85 (s, 9H), 3.02 (br s, 12H). ^{13}C NMR (50.32 MHz, $CDCl_3$) δ: 166.3, 161.8, 161.0, 149.4, 147.9, 129.6, 128.3, 123.3, 121.2, 114.8, 65.0, 56.5, 55.6, 29.9. HRMS ESI$^+$. Calcd for $C_{51}H_{55}N_6O_9$: 895.4032; found $[MH]^+$: m/z = 895.4025. $\mathbf{L^2_{(COOMe)}}$ see Ref. 5.
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15. *Selected spectroscopic data:* **9**. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.03 (s, 3H), 7.85 (s, 3H), 7.45 (d, 6H, J = 8.8 Hz), 6.88 (d, 6H, J = 8.8 Hz), 4.03 (t, 6H, J = 6.2 Hz), 3.96 (m, 15H), 2.96 (br s, 12H), 2.45 (t, 6H, J = 6.4 Hz), 2.26 (s, 18H), 1,97 (m, 6H). ^{13}C NMR (125.76 MHz, $CDCl_3$) δ: 165.7, 161.6, 160.1, 147.5, 133.8, 133.6, 127.9, 125.6, 114.9, 95.6, 85.6, 66.4, 64.4, 56.4, 56.2, 53.1, 45.6, 45.4, 27.5. HRMS ESI$^+$. Calcd For $C_{69}H_{84}N_9O_9$: 394.2142; found $[M+3H]^{3+}$: m/z = 394.2125. $\mathbf{L^3_{(COOMe)}}$. ^{1}H NMR (200 MHz, MeOD) δ: 7.83 (s, 3H), 7.67 (s, 3H), 7.38 (d, 6H, J = 8.9 Hz), 6.93 (d, 6H, J = 8.9 Hz), 4.26 (br s, 4H), 4.16–4.10 (m, 8H), 3.92 (s, 9H), 3.69–3.48 (m, 16H), 3.17 (br s, 12H), 2.94–2.90 (m, 6H), 2.37–2.13 (m, 6H).
16. $[Eu{\cdot}\mathbf{L^3_{(COO)}}]$. HRMS ESI$^+$. Calcd for $C_{75}H_{90}EuN_9Na_4O_{18}S_3$: 436.3593; found $[M+4Na]^{4+}$: m/z = 436.3589.
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