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**PAPER**

# Luminescence of a novel Eu(diketonato)–epoxiphenanthroline complex and covalent coupling to peptides *via* the epoxigroup†

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The application of rare earth complexes in several techniques and technologies requires their strong, *i.e.* preferably covalent, fixation to a substrate. The particularly useful luminescence properties of – readily available – rare earth β-diketonate complexes can widely be maintained on co-ligation with an epoxi-functionalised 1,10-phenanthroline. The linkage of epoxidised 1,10-phenanthroline to common peptides and proteins proceeds to free –SH and $–NH_2$ groups, with a clear preference for –SH. Subsequent luminescence activation with $Eu(ttfa)_3$ yields strongly emissive species, in which the $Eu(ttfa)_3$ coordinates to the *N*-bidentate phenanthroline protruding from the peptide. Luminescence, FTIR, ESI-MS, NMR and life time measurements provide the evidence for successful anchoring of the marker to the peptides. Additionally, the optical properties have been evaluated against analogous Eu complexes in polymer matrices and solution. The procedure described holds the promise of a facile method for the detection of free –SH groups in biological entities and their discrimination from oxidised –S–S– bridges.



## Introduction

The outstanding luminescent properties of rare earth complexes such as aromatic carboxylates and diketonates were recognised several decades ago. Next to persisting fundamental interest, they have received renewed attention more recently in various emerging fields of applications, such as *e.g.* (molecular) organic light emitting diodes,[1] sensors,[2] or bioassays.[3] Particular impact is owed to the event of the commercialization of the DELFIA system,[4] in which the long life emission decay time of many of such complexes is exploited to discriminate the characteristic rare earth emissions against inherent matrix emissions of biological matter. Thus, protein emission signals for example, fading on the nanosecond time scale, can almost completely be ignored, if the rare earth emission is monitored with a time delay of up to several hundred microseconds. Additional factors synergistically contribute to the usability of the rare earths in these applications, among them the narrow emission lines and the large (apparent) Stoke shifts as well as the quantum yields, which can amount to near unity.

Bidentate β-diketonates constitute one class of thoroughly investigated ligands, which led to a well founded understanding of the underlying excitation, energy transfer and emission mechanisms, as summarised and shown in corresponding reviews.[5] In brief, the efficient luminescence of the complexes is facilitated by allowed, thus strong singlet $^1S_0 \rightarrow {}^1S^*$ absorptions, followed by spin–orbit coupling supported, intra-ligand energy transfer to a ligand triplet state $^3T$, from which the energy is transferred to the attached rare earth ion and eventually emitted from the characteristic, well known rare earth excited states, *e.g.* $^5D_0$ in $Eu^{3+}$ or $^5D_4$ in $Tb^{3+}$. Early investigations of rare earth diketonates already revealed the advantageous effect of co-coordination,[6] which essentially removes coordinated water from the luminescent center. If the co-ligands exhibit suitable absorptions, they may additionally contribute to the overall optical properties; popular examples are aromatic chelators such as 1,10-phenanthroline or 2,2′-bipyridine. The eight-coordinate molecular complexes thus typically obtained are soluble in non-polar solvents or may even be evaporated intact. As convenient as this might seem in some instances, for many conceivable applications, it may be necessary however, to link these complexes to a matrix, be it of inorganic, organic, polymeric or biological nature. Thus, we are searching for complexes with versatile, reactive functional groups, readily allowing a covalent affixment to abovementioned matrices.

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† Electronic supplementary information (ESI) available: FTIR spectra of all compounds; ESI MS, $^1H$ and $^{13}C$ NMR spectra and signal assignments for $Na_2$GluSH-phen; absorption and luminescence spectra of Mel-phen and Trigly-phen. See DOI: 10.1039/c2nj40505a

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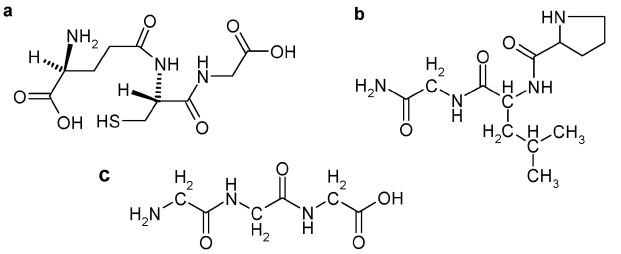



**Fig. 1** Structure of analytes: (a) glutathione ("GluSH"), (b) melanostatine ("Mel"), (c) triglycine ("Trigly").

The present report is devoted to the use of the well known tris(2-thenyl-4,4,4-trifluorbutane-1,3-dionato)Eu ("Eu(ttfa)$_3$"), and 5,6-epoxy-5,6-dihydro-1,10-phenanthroline ("epoxiphen"),[7] as a functional co-ligand. Its capability to bond and label the biologically relevant peptides glutathione ("GluSH"), triglycine ("Trigly"), and melanostatine ("Mel") is elucidated (see Fig. 1). Next to their presumed model character for the more cumbersome and complex reactions with proteins, GluSH, exhibiting free –SH groups, was chosen for its vital role as an antioxidant, and its indicator function for cellular toxicity,[8] Trigly for the absence of –SH groups, and Mel, a melanin inhibitor, for its amide function and its exclusively secondary amine groups.[9]

## Results and discussion

### Reactions of epoxiphen (5,6-epoxy-5,6-dihydro-1,10-phenanthroline) with peptides

The expected reaction of epoxiphen with polar groups like amino and thiol, respectively, would generally proceed according to eqn (1a) or eqn (1b).[10,11]

The progress of the reaction can readily be followed by observing the disappearance of the asymmetric stretch of the epoxy C–O–C vibration in the infrared spectra (IR, 882 cm$^{-1}$, see Fig. S1b, ESI†). To confirm this assignment, a model reaction was carried out with glutathione and butylamine at pH = 4, the product of which was practically void of COC vibrations in the spectral range of interest (Fig. S1g, ESI†).

The amine coupled reaction products 1a can in fact be identified for Trigly and Mel. However, a relatively high pH of 10 was required, while at a pH of 4, no reaction could be observed at all. At intermediate pH values the reactions rates are drastically reduced, such that unreacted epoxiphen could still be detected even after a reaction period of 3 days.

As opposed to that, the thiole linkage appears to be preferred, as was demonstrated using GluSH, where the epoxiphen has completely vanished after 50 hours even at a pH as low as 4.

The corresponding UV absorption spectra for the reactions of GluSH at pH 4 and 10 are reproduced in Fig. 2 along with the starting materials. At pH values of >9 the disappearance of the H–S– valence mode (2526 cm$^{-1}$) in the IR spectrum serves as an additional monitor for detection, furthermore, a slight shift of epoxiphen bands is observed (Fig. S1e, ESI†), while products related to eqn (1a) (amine linkage) are not. Next to mentioned epoxy C–O–C vibrations, the solid epoxiphen ligand itself can be excited in the UV (250–400 nm) to exhibit a detectable emission in the blue to green spectral region, the

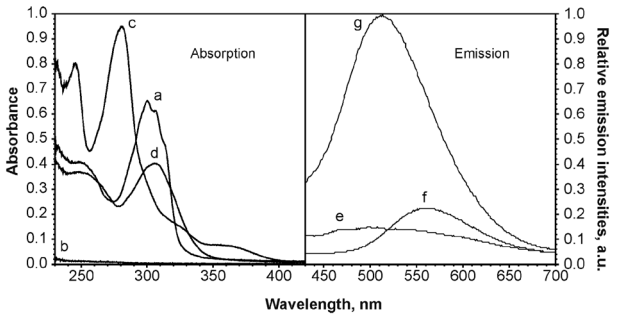
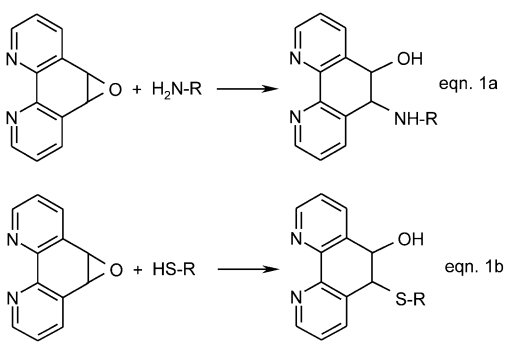



**Fig. 2** Left: absorption spectra ($5 \times 10^{-5}$ mol l$^{-1}$) of (a) epoxiphen, solids of (b) GluSH, (c) $H_2$GluS-phen (pH = 4), (d) $Na_2$GluS-phen (pH = 10). Right: emission spectra of (e) epoxiphen, (f) $H_2$GluS-phen, (g) $Na_2$GluS-phen.

exact location of which appears to be depending on the ligand's substituents, *i.e.* –NH–R or –S–R (see Fig. S4 also, ESI†). Qualitatively, the ligand emission spectra are blueshifted with increasing electron richness. Accordingly, the emission of the thiol coupled di-anion (the di-sodium salt, "$Na_2$GluS-phen") is also blueshifted by approximately 50 nm, and, additionally, its emission intensity is enhanced considerably in the di-sodium salt over the free acid (Fig. 2).

Further evidence for the formation of the thiol-linked epoxiphen (eqn (1b), Scheme 1) can be derived from the corresponding MS and NMR spectra. In the ESI-MS in particular, significant fragmentation reactions are water releases and a characteristic cleavage of the fragment $H_2NCH(CH_2S$-phen$)CONHCH_2COOH$ ($m/z$ = 357 Da) (see Fig. S2, ESI† for further details). Because of the high ionization efficiency of the phen-group, several corresponding fragment ions appear, indicating the sulfur linkage. In contrast, bonds to oxygen or nitrogen would generate two other pairs of characteristic fragment ions each (215 and 197 or 214 and 196, respectively). Compared with this, MS spectra obtained for the amine based reaction of epoxiphen with Mel and Trigly indicate the presence of the amine linkage (Scheme 1, eqn (1a)), however, the Mel and Trigly materials were obviously strongly contaminated with unidentified by-products and starting materials, respectively, and were not pursued further (for completion, their absorption and emission spectra are depicted in Fig. S4, ESI†). Finally, the NMR spectra agree with Fig. 1b for the glutathione substitution also (see Experimental section). In addition the $^{13}$C NMR data suggest the presence of two conformers in solution.




**Scheme 1** Reaction pathways for coupling of epoxiphenanthroline.

### The complex Eu(ttfa)$_3$epoxiphen

While the preparation of epoxiphen, its conversion into aminophenanthroline and the successive complexation with $Eu^{3+}$ to yield Eu(ttfa)$_3$aminophen[13,14] has been reported, the complex Eu(ttfa)$_3$epoxiphen (Fig. 3) and its spectral features have to the best of our knowledge not been described before. This is somewhat surprising, because the epoxiphen co-ligand itself is easily accessible[7] (or even available commercially), grants a ready straightforward access to Eu(ttfa)$_3$epoxiphen, which is in turn a very interesting complex due to the reactive epoxigroup. The co-ligand thus provides an elegant coupling substituent for numerous applications, in which covalent linkage to substrates is mandatory such as in sensors, markers, *etc.*

Eu(ttfa)$_3$epoxiphen proved to retain its spectral features and efficiency in comparison to the 'original' parent complex Eu(ttfa)$_3$phen (quantum yields $\Theta$ of 66% compared to 82% and corresponding lifetimes of 864 μs and 945 μs, respectively, we should mention here that other data reported for Eu(ttfa)$_3$phen amount to 69% and 976 μs, respectively,[15] and 82%,[16] see Table 1).

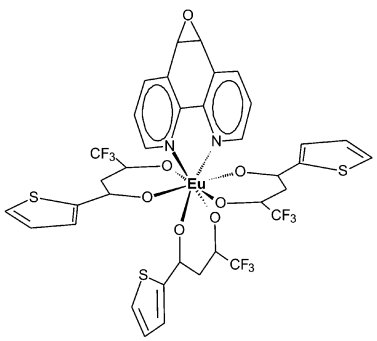



**Fig. 3** Sketch of Eu(ttfa)$_3$epoxiphen. Structural details of the parent Eu(ttfa)$_3$phen may be found in ref. 12.

**Table 1** Luminescence properties of Eu(ttfa)$_3$epoxiphen and GluSH-coupled complexes: excitation and emission maxima ($\lambda_{exc}$, $\lambda_{em}$), luminescence lifetimes ($\tau$) and quantum yields ($\Theta$) in comparison to other Eu(ttfa)$_3$ containing species

| Sample | $\lambda_{exc}$ (nm) | $\lambda_{em}$ (nm) | $\tau$ (μs) | $\Theta$ (%) |
|---|---|---|---|---|
| Eu(ttfa)$_3$epoxiphen | 360 | 612 | 864 2 | 66[a] |
| Eu(ttfa)$_3$phen | 380 | 612 | 945 11 | 82[a,b], 69[c] |
| Eu(ttfa)$_3$(H$_2$O)$_2$ | 375 | 610 | 198 1 | 23[c] |
| Eu(ttfa)$_3$GluSH | 340 | 612 | 266 1[a] | — |
| Eu(ttfa)$_3$GluS-phen | 360 | 612 | $\tau_1$: 162 11 (8%)<br>$\tau_2$: 494 8 (92%) | 20.6 |
| Eu(ttfa)$_3$bu-aminophen[d] | 360 | 612 | 585 1[a] | — |
| Eu(ttfa)$_3$aminophenMR[e] | 349 | 612 | 500 | 22 5[f] |
| Eu(ttfa)$_3$phen/PMMA | 360 | 612 | 552 2 (spheres) | 35[a] |
| Eu(ttfa)$_3$/PMMA | 350 | 615 | 59 1 (20%)<br>362 3 (80%) | 0.6[a] |
| Eu(ttfa)$_3$phen/silicone | 365 | 612 | 526 1 | 8 |
| Eu(ttfa)$_3$phen/PUR | 365 | 615 | 601 1 | 36.7[a] |
| Eu(ttfa)$_3$/PUR | 380 | 614 | 558 1 | 17.6 |

[a] This work. [b] Ref. 16. [c] Ref. 15. [d] From reaction of epoxiphen with butyl amine prior to complexation. [e] MR = Merrifield resin. [f] Ref. 13. PUR = polyurethane (from hexamethylenediisocyanate and polyol),[21] PMMA = polymethylmetacrylate.

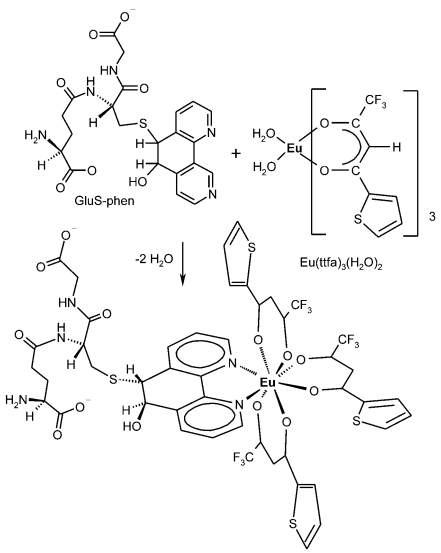
### Functionalization of the epoxiphen coupled glutathione (GluS-phen) with Eu(ttfa)$_3$

The final functionalization of the epoxiphen-modified glutathione with Eu(ttfa)$_3$ readily proceeds in a water–ethanol solution within a few hours (Scheme 2). Again, despite some band broadening, IR is a good tool for monitoring characteristic changes of the starting materials in the reaction.

IR absorptions of Eu(ttfa)$_3$ and epoxiphen at 1580, 1560, 1433, 1216, 1013 cm$^{-1}$ are shifted to higher frequencies on co-complexation according to Fig. S1 (ESI†). The very same effects are also observed on co-complexation of GluS-phen to the europium diketonate. While on co-coordination of epoxiphen the characteristic 751 cm$^{-1}$ absorption band (out-of-plane bending modes of the hydrogen atoms) is red shifted and split into a doublet at approximately 744 and 721 cm$^{-1}$, in GluSH-phen the corresponding 749 cm$^{-1}$ band appears as a doublet at 746 and 720 cm$^{-1}$ on coordination to Eu(ttfa)$_3$. These findings are in complete agreement with the spectra reported for the co-coordination of 1,10-phenanthroline in similar complexes, *e.g.* Eu(O$_2$C–CH=CH–C$_6$H$_5$)$_3$phen[17] or Eu(NO$_3$)$_3$(phen)$_2$.[18] Additionally, we observe redshifts of the phen absorptions at 800 cm$^{-1}$ and 707 cm$^{-1}$ to 786 cm$^{-1}$ and 682 cm$^{-1}$ on Eu-coordination and from 709 cm$^{-1}$ to 682 cm$^{-1}$, respectively, in the corresponding Eu(ttfa)$_3$(GluS-phen). The coordination of epoxiphen or GluS-phen also gives rise to a slight redshift from 1544 to 1539 cm$^{-1}$ of the perturbed diketonate carbonyl groups of the ttfa ligand (Fig. S1, ESI†).

Ultimately, spectroscopic evidence for the presence of the linkage between the Eu(ttfa)$_3$ and the modified glutathione is provided by the spectral and lifetime analysis of the Eu emissions lines. The $Eu^{3+}$ emission is known to be particularly sensitive to the chemical environment in that the presence of water or other entities exhibiting high vibrational frequencies in the first coordination sphere, inevitably lead to radiationless decays, *i.e.* reduced lifetimes of the excited states and reduced efficiencies.

Typically, 1,10-phenanthroline and other bidentate co-ligands to the diketonates provide very good screening and thus




**Scheme 2** Formation of Eu(ttfa)$_3$GluSH-phen.

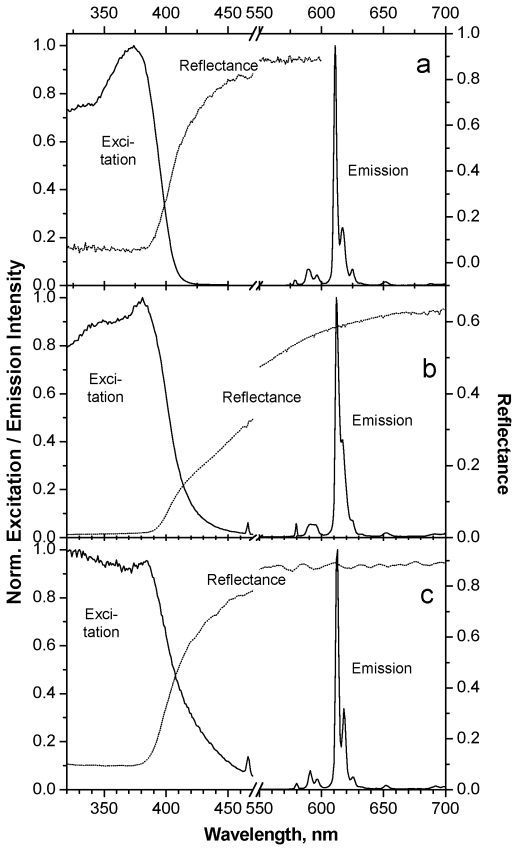



**Fig. 4** Reflectance and normalised excitation and emission spectra of (a) Eu(ttfa)$_3$phen ($\lambda_{exc}$ = 380 nm), (b) Eu(ttfa)$_3$(GluS-phen) ($\lambda_{exc}$ = 360 nm) and (c) Eu(ttfa)$_3$epoxiphen ($\lambda_{exc}$ = 360 nm). Emission monitored at 612 nm. The non-normalized emission intensity in Eu(ttfa)$_3$(GluS-phen) amounts to *ca.* 25% and 30% of Eu(ttfa)$_3$phen and Eu(ttfa)$_3$epoxiphen.

enable high efficiencies, often in excess of 80%, which are accompanied by decay times up to the millisecond regime.

A comparison of the emission spectra of Eu(ttfa)$_3$phen, Eu(ttfa)$_3$epoxihen and Eu(ttfa)$_3$(GluS-phen) is enabled in Fig. 4. The emission pattern of Eu(ttfa)$_3$(GluS-phen) resembles that of Eu(ttfa)$_3$phen; the line broadening is analogous to that observed for attachment to the Merrifield resin. Thus the luminescence spectra support the assumption of structural analogy in the immediate $Eu^{3+}$ coordination sphere. Further, Table 1 allows a quantitative comparison of the optical properties of the present Eu(ttfa)$_3$epoxiphen and Eu(ttfa)$_3$(GluS-phen) with the parent compounds Eu(ttfa)$_3$ and Eu(ttfa)$_3$phen as the pure compound and some matrix embedded materials.[19,20] The increase of the radiative lifetimes of the $Eu^{3+}$ ions on co-coordinating GluS-phen to Eu(ttfa)$_3$ and the measured quantum yields are in good agreement with results found for mentioned phen-functionalised Merrifield resin (polystyrene)[13] and generally in line with the decay depreciation brought about by secondary interactions of the complexes with "high frequency matrices" (–C–H vibrations of polymer matrices or other species, see Table 1). The GluS-phen ligand furthermore introduces a second lifetime component, albeit of weak amplitude (8%) only, the assignment of which is not unambiguous at present. We speculate that a partial substitution of ttfa$^-$ by a GluS-phen carboxylate group due to the higher basicity of the ttfa$^-$ ligand may have taken place, a behaviour that we have previously observed at pH < 7 with other carboxylic acids. The formation of trace amounts of a Schiff-base from the GluS-phen aminofunctions and ttfa, analogous to β-diketonates of $Cu^{2+}$ and $VO^{2+}$,[22,23] should also not be ruled out.

## Summary and conclusions

Well known diketonato complexes of $Eu^{3+}$ can readily be co-coordinated with the bidentate co-ligand 5,6-epoxy-5,6-dihydro-1,10-phenanthroline. The resulting luminescent complexes are thus enabled to undergo covalent coupling reactions with a multitude of conceivable reaction partners. However, in a series of reactions with amines and thiols we found a marked preference for coupling *via* –SH groups. It proved to be advantageous, to react the co-ligand with the epoxiphen prior to the eventual luminescence activation with the Eu diketonate complex Eu(ttfa)$_3$. Consequently, the reaction of epoxiphenanthroline with *e.g.* glutathione at moderate pH values exclusively leads to a sulfur linkage to the phenanthroline. The modified glutathione obtained can readily be functionalised optically with europium diketonates, such as Eu(ttfa)$_3$, and thus be rendered luminescent. The scheme described holds the promise of becoming a sensitive optical sensor for glutathione, but possibly other biologically relevant molecules as well. As an example, the reduced form of BSA may be mentioned here, to which we were able to couple the complex as well. The epoxy complexes and the gluthathione derivatives were compared to known standard europium luminophores and found to be in a very promising efficiency regime optically. In future work, we will try to optimise the synthesis and investigate the detection limits for glutathione in particular. However, due to its capability of covalent binding, the complex is also of interest for the facile synthesis of inorganic–organic hybrid materials, *i.e.* for backbone anchored luminescent complexes within polymers, which will be a second focus in coming research.

## Experimental section

### General experimental methods

IR spectra were recorded on Perkin Elmer Spectrum One FTIR spectrometer as KBr disks in the range between 4000 cm$^{-1}$ and 450 cm$^{-1}$. A discussion of the relevant spectral features is conducted in the text. Given the multitude of absorption bands, a listing shall here be refrained from; instead, spectra are reproduced along with a comparison of relevant analogs in Fig. S1 (ESI†).

Mass spectroscopic experiments were carried out on an Agilent 1100 series binary HPLC system (Agilent Technologies, Waldbronn, Germany) coupled with a 4000QTRAPTM linear ion trap mass spectrometer (Applied Biosystem/MDS SCIEX, Foster City, CA, USA) equipped with a TurboIon spray source. Separation was achieved on a ProntoSIL 120-C18-SH (Bischoff Chromatography, Leonberg, Germany) column (150 × 2 mm i.d., 3 μm particle size) kept at 20 °C during analysis. Gradient elution was done with deionised water with 0.1% formic acid

(solvent A) and acetonitrile with 0.1% formic acid (solvent B) at a constant flow rate of 300 µl $min^{-1}$. The gradient profile was 1% B for 5 min isocratic, from 5 to 25 min a linear increase from 1% B to 99% B, an isocratic step for 10 min and a return to 1% B at 26 min and 10 min isocratic for re-equilibration. The injection volume was 15 µl. The MS was operated in the positive Enhanced MS (EMS) mode ion scanning from 100–700 nm. The parameters used for all methods were optimised performing a Flow Injection Analysis (FIA) with standards and led to the following settings for all analyses: curtain gas ($N_2$) 10 arbitrary units (au), temperature of the source 400 °C, nebuliser gas ($N_2$) 50 au and heater gas ($N_2$) 20 au. For compound identification MS/MS and MS/MS/MS experiments were performed in Enhanced Product Ion (EPI) Scan mode. Collision Energy (CE) was optimised individually for each precursor ion.

$^1$H and $^{13}$C NMR spectra of $Na_2$GluS-phen were measured on an AVANCE I (Bruker) 400.13 MHz NMR spectrometer equipped with a BBO(F) sample head with a *z*-gradient. The sample was dissolved in $D_2O$ at a concentration of 0.187 mol $l^{-1}$, 3-(trimetylsilyl)propane sulfonic acid sodium salt (TSPSA) was used as an internal standard (chemical shift 0.015 ppm). $^{13}$C spectra are CH-decoupled.

UV-Vis absorption spectra were obtained using an Ocean Optics HR4000 fibre spectrometer with a 5 µm slit and a 20 W deuterium/halogen light source. Emission and excitation spectra of the powderous products were measured at room temperature on an ARC spectrometer (monochromators of 300 mm focal length, a 450 W Xe lamp as a light source, photomultiplier tube P2) equipped with optical fibres. The quantum yields were obtained by measuring relative to a Lumogen Red F300 doped (50 ppm) PMMA powder standard, for which a quantum yield of 42% was assumed.

Luminescence decay times were measured using an Edinburgh Instruments FL 920 lifetime spectrometer (single photon counting) equipped with an Edinburgh Instruments µF900 flash lamp and a Hamamatsu extended red sensitivity photomultiplier tube.

### Synthesis

**5,6-Epoxy-5,6-dihydro-1,10-phenanthroline (epoxiphen).** Epoxiphen was synthesised according to Shen and Sullivan[7] with the exception that the formation of epoxiphen was monitored *via* IR spectroscopy. The spectra were identical to a commercial product purchased from Aldrich.

**Coupling of GluSH with (epoxiphen).** 200 mg GluSH (0.651 mmol, Aldrich) were dissolved in 10 ml dest. water and mixed with 120 mg 5,6-epoxy-5,6-dihydro-1,10-phenanthroline (0.612 mmol), dissolved in 2 ml methanol. The mixture was stirred at room temperature for at least 20 h. The solvent was evaporated under vacuum ($10^{-3}$ mbar) at room temperature. The yellow powderous product was washed several times with toluene. The toluene contained no residue after evaporation, if the reaction time exceeded 40 h.

In ESI-MS, the signal of highest mass at 405 Da corresponds to the quasi molecular ion $[M + H]^+$ of composition $C_{22}H_{26}N_5O_7S^+$. The complete fragmentation scheme is given in Scheme S2 (ESI†). For a discussion of relevant IR vibrations see text (further spectral information in Fig. S1, ESI†).

**$Na_2$GluS-Phen.** 200 mg GluSH (0.651 mmol) were dissolved in 10 ml dest. water, the solution was adjusted with 2 M NaOH solution to a pH of 9, and mixed with 127.4 mg 1,10-phenanthroline-5,6-epoxide (0.65 mmol) dissolved in 2 ml methanol. The mixture was stirred at room temperature for 20 to 45 h, after which the solvents were evaporated under vacuum ($10^{-3}$ mbar) at room temperature. The slightly yellowish powder was washed several times with toluene (at 45 h reaction time no residue was observed after evaporation of toluene). For the reaction at pH $\approx$ 9 of GluSH with epoxiphen, the highest signal in MS appears at $m/z$ = 504, corresponding to $[GluS\text{-}phen + H]^+$ ($C_{10}H_{16}N_3O_6S$–$HOC_{12}H_9N_2$) (see text, Fig. S1 and Scheme S1, ESI†). In NMR all signals can be assigned to the product indicated by eqn (1b), albeit in $^{13}$C, the appearance of doublets (some only under very strong magnification) suggests the presence of two fairly stable conformers in the solution:

$^1$H NMR $\delta_H$ 1.88–1.87 (d, 2H); 2.37 (s, 2H); 2.805–2.92 (m, 1H); 3.1–3.32 (m, 1 H); 3.28 (s, 1H); 3.80 (d, 2H); 4.56 (s, 1H); 4.60 (d, 1H); 5.18 (d, 1H); 7.5–7.6 (m, 2H); 8.00 (dd, 2H); 8.70 (d, 1H); 8.75 (d, 1H). $^{13}$C NMR $\delta_H$ 33.24 (s); 34.91 (s); 35.20, 35.30 (d); 46.06 (s); 49.37, 49.88 (d); 55.13, 55.71 (d); 58.0 (s); 72.07, 72.35 (d); 127.95, 128.0 (d); 128.20 (s); 134.15, 134.27 (d); 134.41 (s); 141.55, 141.65 (d); 151.22, 151.23 (d); 151.45, 141.48 (d); 152.18, 152.27 (d); 153.1 (s); 174.11, 174.15 (d); 178.77, 178.8 (d); 178.9 (s); 184.22, 184.26 (d). The signals for GluSH strongly depend on pH. The assignment summarised in Table S1 (ESI†) follows previous assignments on GluSH.[24,25]

**Eu(ttfa)$_3$(epoxiphen).** $Eu(ttfa)_3(H_2O)_2$ was prepared according to Charles and Ohlmann.[26] The epoxiphen-cocomplex was subsequently obtained by dissolution of 0.851 g $Eu(ttfa)_3(H_2O)_2$ (1 mmol) in 15–20 ml ethanol. 0.196 g epoxiphen were dissolved in 10 ml ethanol and added to the previous solution and the mixture left overnight at room temperature for the complex crystallization. The powder was filtered off, washed with *n*-hexane and dried under vacuum at room temperature. Analytical data for $C_{36}H_{30}N_2O_3S_3F_9Eu$: Eu 15.02%, found 15.06%; C 42.74%, found 43.10%; S 9.51%, found 9.25%.

**Eu(ttfa)$_3$($Na_2$GluS-phen).** 200 mg $Na_2$GluS-phen (0.364 mmol) were dissolved in 2 ml dest. water and 7 ml ethanol. 310 mg $Eu(ttfa)_3$ were dissolved in 1 ml ethanol and added to the conjugated tripeptide solution. The precipitate formed was heated for 9 h at 50 °C with an additional 7 ml of ethanol. After cooling the precipitate was centrifuged and washed several times with ethanol to remove non-reacted $Eu(ttfa)_3$. Yield 30%. Analytical data for $Na_2C_{46}H_{35}N_5O_{13}S_4F_9Eu$: Eu 11.15% (found 11.10%), C 40.54% (found 40.72%), S 9.38% (found 9.40%).

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