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Structure Elucidation

Site Covalent Modification of Methionyl Peptides for the Production of FRET Complexes

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Abstract: Flax cyclic peptides (orbitides, linusorbs (LOs)) [1–8-NαC],[1-$MetO_2$]-linusorb B1 ([$MetO_2$]-LO1) and [1–9-NαC],[1-$MetO_2$]-linusorb B2 ([$MetO_2$]-LO2) are biologically active. These LOs lack active nuclei commonly used in peptide modification. We have developed reactions to activate methionine methyl sulphide to produce stable derivatives. In these reactions, LOs are converted to sulfonium intermediates and subsequently to derivatives containing active nuclei while preserving their fundamental structures. The reaction conditions preserved cyclic peptide fundamental structure and organic solvent solubility. [Met]-LO1 and [Met]-LO2 analogues containing activated groups (–CN, –COOEt, and $–NH_2$) in the form of methionine, methionine (*S*)-oxide, and methionine (*S*,*S*)-dioxide amino acids were synthesized and characterized by LCMS and 1D and 2D NMR spectroscopy. Coumarin orbitide complexes produced in this manner bind Eu^{3+} yielding FRET compounds that absorb energy through coumarin antennae and emit photons at lanthanide wavelengths.

Introduction

Lanthanide complexes are used in various biomedical applications,[1] supramolecular chemistry,[2] and structural analysis.[3] Lanthanide ions, Ln^{3+} show interesting luminescent properties with sharp emission peaks, long luminescence lifetimes (microsecond to milliseconds), large Stoke shifts and emissions ranging from visible to near-IR.[4] Lanthanides have been incorporated into biomolecules such as peptides at calcium binding sites,[5] attached at reactive side chains like cysteine,[6] or coupled to modified amino acids.[7] However the inherent drawback of Ln^{3+} ions is low quantum yield, which can be overcome in part by coupling Ln^{3+} with fluorophores such as tryptophan or other fluorescent compounds.[7,8] Macrocyclic cryptand molecules such as crown ethers have been used as substrates with coumarin as sensitizers. Molecules with greater rigidity lose less energy in nonradiative emission.[9]

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Lanthanide binding peptide ligands have been synthesized by solid-phase peptide synthesis and used for fluorescence studies in aqueous buffer solutions. Thermodynamic stability is necessary if these compounds are to be used for in vitro and in vivo applications.[10] Cyclic peptides and particularly orbitides are more stable than linear peptides with the same sequence and they typically resist protease degradation.[11] Cyclic peptides with chromophores can be prepared using solid-phase supports[12] or direct modification at reactive side chain amino acids including serine, threonine, cysteine, and lysine.[13] The latter approach is particularly efficient for peptides that have few heteroatoms and, therefore, can be converted without the use of protective groups. A further advantage of adduct formation is realized with mild reactions that preserve peptide chirality. This approach can help to generate families of analogues that are suited for studies of structure/function activity relationships of biologically active peptides.[14]

The systematic alkylation of methionine residues was previously reported for proteins using α-halo acetic acids and related derivatives.[15] This reaction yielded a sulfonium salt, which was converted by heating at 100 °C to either homoserine or homoserine lactone in which the product arises from the reaction of a C-terminal methionine residue. It was also reported that methionine can be regenerated from sulfonium salts using β -mercaptoethanol.[16] This approach was used for isolation of methionine peptides under acidic conditions.[17] The synthesis of sulfonium polypeptides is also possible through methionine alkylation.[18]

Flax seed oil (*Linum usitatisssimum* L.) contains hydrophobic orbitides (linusorbs, LOs) comprising eight to ten amino acids. To date, 28 LOs, with molecular weights of approximately 1 kDa have been isolated from flaxseed.[19] Glycine and trypto-

Table 1. Amino acid sequences of glycine and tryptophan free LOs (**1**–**7**).

LO[a]	Previous names	Amino acid sequence[b]	Molecular formula
[1–9-NαC]-linusorb B3 (**1**)	CLA	NαC-(Ile-Leu-Val-Pro-Pro-Phe-Phe-Leu-Ile)	$C_{57}H_{85}N_9O_9$
[1–9-NαC]-linusorb B2 (**2**)	CLB	NαC-(Met-Leu-Ile-Pro-Pro-Phe-Phe-Val-Ile)	$C_{56}H_{83}N_9O_9S$
[1–9-NαC],[1-(R_s,S_s)-MetO][c]-linusorb B2 (**3**)	CLC	NαC-(MetO[d]-Leu-Ile-Pro-Pro-Phe-Phe-Val-Ile)	$C_{56}H_{83}N_9O_{10}S$
[1–9-NαC],[1-$MetO_2$]-linusorb B2 (**4**)	CLK	NαC-($MetO_2$[d]-Leu-Ile-Pro-Pro-Phe-Phe-Val-Ile)	$C_{56}H_{83}N_9O_{11}S$
[1–8-NαC]-linusorb B1 (**5**)	CLP	NαC-(Met-Leu-Val-Phe-Pro-Leu-Phe-Ile)	$C_{51}H_{76}N_8O_8$ S
[1–8-NαC],[1-(R_s,S_s)-MetO]-linusorb B1 (**6**)	CLE	NαC-(MetO-Leu-Val-Phe-Pro-Leu-Phe-Ile)	$C_{51}H_{76}N_8O_9$ S
[1–8-NαC],[1-$MetO_2$]-linusorb B1 (**7**)	CLJ	NαC-($MetO_2$-Leu-Val-Phe-Pro-Leu-Phe-Ile)	$C_{51}H_{76}N_8O_{10}$ S

[a] Linusorbs naming based on the proposed nomenclature of orbitides.[19d] [b] Homodetic cyclic peptides with N to C linkage between first and last amino acid nomenclature convention.[22] [c] Methionine (*S*)-oxide has a chiral *S*. Epimers are designated *S* and *R*. [d] Designation of MetO and $MetO_2$ describe methionine (*S*)-oxide and methionine (*S*,*S*)-dioxide.

phan-free LOs are shown in Table 1. LOs **1** (LO3), **2** (LO2), **3** ([MetO]-LO2), and **6** ([MetO]-LO1) have shown in vitro antitumor activity against different cancer cell lines.[20] LO **1** inhibits both cholate uptake into hepatocytes of rat liver (50% inhibition at 3 μm) and T-lymphocyte activation and proliferation. Related compounds such as somatostatin and antanamide also inhibit cholate uptake into hepatocytes.[21]

LO **1** inhibits concanavalin A induced T-cell proliferation (IC_{50} of 2.5 $\mu g\,mL^{-1}$) with the largest immunosuppressive activity on human peripheral blood lymphocytes of LOs tested. Flax LOs, ([$MetO_2$]-LO2) (**4**) and ([$MetO_2$]-LO1) (**7**) also possess immunosuppressive activity (Table 1).[23] The IC_{50} of **4** (25.2 $\mu g\,mL^{-1}$) and **7** (28.1 $\mu g\,mL^{-1}$) occurred at higher concentrations.[23] LOs **4** and **7** were synthesized in 75% isolated yield by oxone oxidation of **3** and **6**, respectively,[24] but were also detected in flax meal heated at 100 °C for 16 h and silica that has been in contact with flax oil.[25]

The biological activity of **1** might arise from the Pro-Phe-Phe tripeptide moiety. It was also found that the amino acid triad Pro-Xxx_1-Xxx_2 (in which Xxx_1 and Xxx_2 are either hydrophobic or aromatic amino acid residues) was also present in other immunosuppressant cyclic peptides such as cycloamanide B (Pro-Ser-Phe), antamanide (Pro-Phe-Phe) and hymenistatin I (Pro-Tyr-Val).[21a,26] Similar tripeptide fragments also occur in **4** and **7** in which Xxx_1 is Phe in **4**, but Leu in **7**. Hence chemical modifications of **4** and **7** might display this bioactive tripeptide fragment if these amino acids remain intact and separated from the modified site.

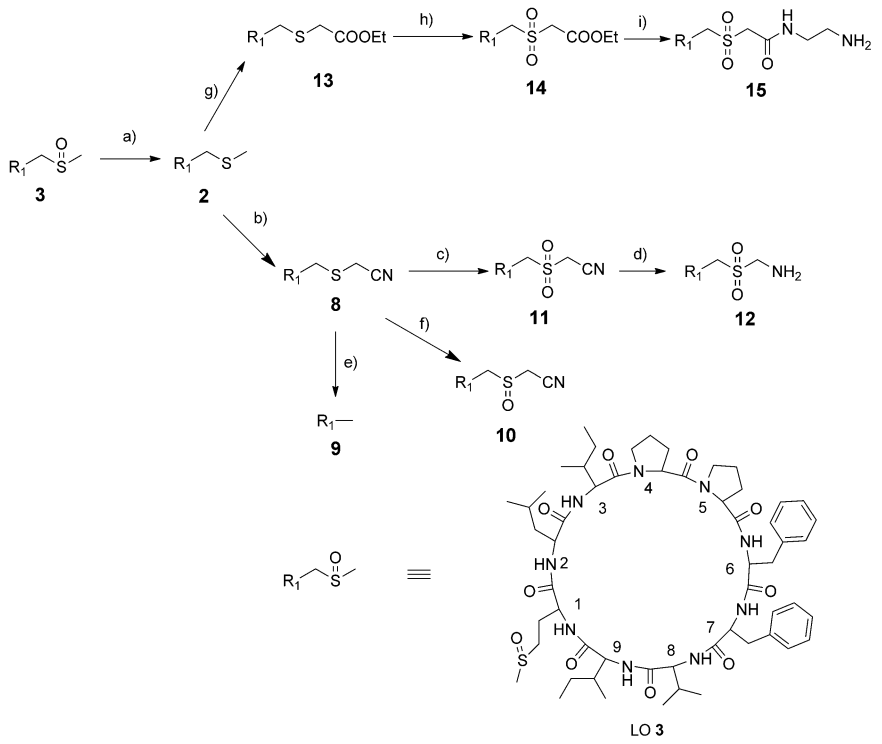
LOs could be utilised as drugs or drug delivery agents as they have biological activity, hydrophobicity and may form inclusion complexes.[27] LOs contain intrinsic fluorophores such as Phe and Trp in their cyclic structure. LO **1** has been reported to transfer energy to Tb^{3+} through Phe fluorescence.[27b] However natural amino acid fluorophores have limitations as excitation occurs at wavelengths where background absorbance is high for both the excitation and emission wavelengths, this would make detection of a fluorescent signal in living tissues difficult. Attaching a fluorescent dye to an orbitide would overcome this but there are no prior reports of attachment of fluorescent dyes to LOs.

LOs contain amino acids such as Trp and Met that may be suited for attachment of fluorescent dye or for producing other modifications. This paper describes site-specific modification of LOs **3** and **6** through methionine residues while maintaining their fundamental structure. To our knowledge modification of cyclic peptides and LOs through methionine has not been previously reported. A general synthetic protocol was developed that was used to prepare fluorescent LO adducts from commercially available dyes. Spectroscopic analysis of fluorescent adducts was conducted in different solvents to understand the photophysical behaviour of coumarin LO complexes. LO conjugated coumarin complexes were also coupled with transition metals to be used in LRET (lanthanide resonance energy transfer) analysis. In addition, these modified LOs have been characterized using spectrometric techniques including 1D and 2D NMR. Hence LOs tagged with a fluorescent label can potentially be used in therapeutic monitoring and fluorescence energy transfer.

Results and Discussion

LOs **4** [NαC-($MetO_2$-Leu-Ile-Pro-Pro-Phe-Phe-Val-Ile)] and **7** [NαC-($MetO_2$-Leu-Val-Phe-Pro-Leu-Phe-Ile)] are biologically active[23] and heat stable.[25] Attempts to reduce $MetO_2$ to Met were unsuccessful (data not shown). The Met containing LOs **2** and **5** were, therefore, accessed through reduction of methionine (*S*)-oxide (MetO) precursor LOs **3** and **6**, respectively. MetO containing LOs were readily reduced to Met containing peptides using (NH_4I/TFA/Me_2S) and ($TiCl_4$/NaI).[28] Modification of resulting Met LOs with reactive iodomethylene groups is then possible.[15a] Met, therefore, serves as point of attachment for an activated side chain while keeping the fundamental structure intact. Peptides containing reactive groups such as –NH_2, –COOH, and –OH may thus be synthesized to allow selective coupling with other reactive functional groups present in fluorescent labels.

LO **3** was reduced to **2** by using sodium borohydride in presence of iodine (Scheme 1).[29] Met is reactive and can be reacted with various electrophilic groups such as haloalkyl or haloacetyl to form additional compounds.[18a] The plausible mechanism for Met alkylation with an electrophilic group (R–X) is through formation of a sulfonium intermediate and subsequently release of MeX as a leaving group to form a S–R compound.[15a] LO **2** was subsequently treated with iodoacetonitrile in the presence of DMF and *N*-methylpyrrolidone at 100 °C for 20 h to form the nitrile, **8** (–SCH_2CN). Similar treatment with io-



Scheme 1. Synthesis of amine derivative **15**: a) $NaBH_4$, I_2, THF, RT; b) ICH_2CN, DIPEA, NMP, 100 °C; c) *m*CPBA, DCM, RT; d) H_2 (1 atm.), PtO_2, AcOH, RT; e) H_2 (1 atm), Pd/C, LiOH, dioxane/water, 140 °C; f) $NaIO_4$, THF: H_2O; g) ICH_2COOEt, DIPEA, NMP, 100 °C; h) *m*CPBA, DCM, RT; i) ethylenediamine, MeOH, reflux. *m*CPBA = *meta*-chloroperbenzoic acid; DIPEA = *N*,*N*-diisopropylethylamine.

doacetate yielded the ester, **13** (–SCH_2COOEt). Met compounds can be eliminated using Raney Ni to afford alkanes (α-amino butyric acid)[30] or to produce an alkene at elevated temperature ((*S*)-vinyl glycine).[31] In our attempts to reduce nitrile **8** to an amine led to elimination of mercaptoacetonitrile or hydrogen disulfide to form **9** under the experimental conditions.[32] To avoid producing the elimination product, **8** was oxidized to (*S*)-oxide nitrile (**10**) (–$SOCH_2CN$). However, the (*S*)-oxide nitrile **10** underwent elimination under reduction conditions (H_2 at 1 atm., Pd–C, and LiOH at 170 °C) to produce mixtures of external and internal olefins (data not shown). It was surmised that oxidation of **8** to a more stable (*S*,*S*)-dioxide **11** (–SO_2CH_2CN) will provide access to an amine **12** that resists elimination. Thus, *m*CPBA oxidation of **8** yielded **11**, which was reduced to amine **12** (–$SO_2CH_2CH_2NH_2$) under 1 atm. of H_2, PtO_2, and AcOH at room temperature in low yield. Elimination products were not detected under these reaction conditions.

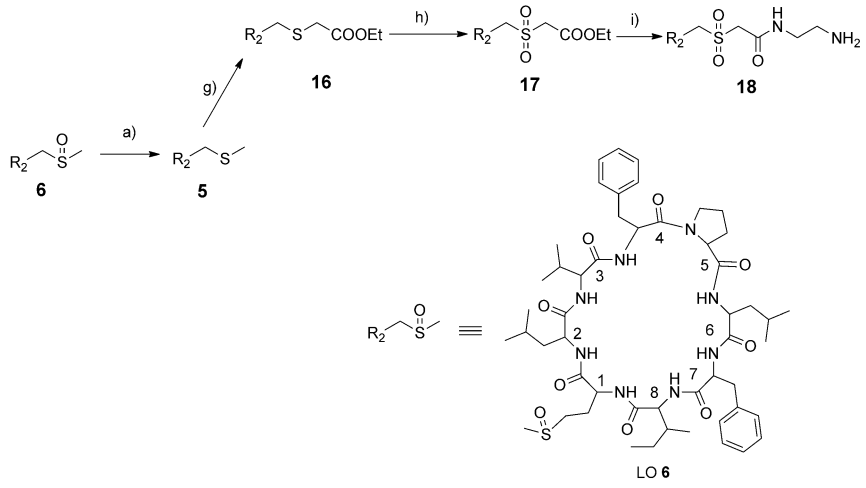
An alternative and better yielding route to amine **15** that does not lead to elimination products was sought. Amine **15** was obtained from ester **13** by following its oxidation to a comparatively thermally stable **14**. Treatment of **14** with ethylene diamine provided access to amine **15** in 86% isolated yield. Following successful synthesis of amine **15** (Scheme 1), amine **18** was similarly prepared from LO **6** (Scheme 2).

LO fluorescent adducts

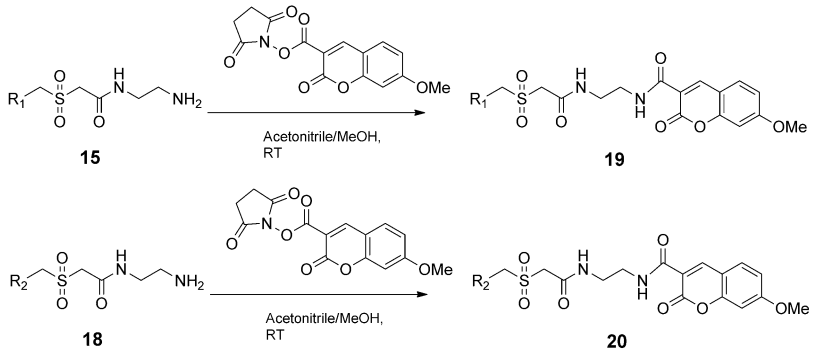
Amines **15** and **18** (Scheme 1 and 2) were used to prepare fluorescent LOs **19** and **20**, respectively (Scheme 3). The 7-methoxycoumarin-3-carboxylic acid succinimidyl ester was prepared as previously reported[33] and was coupled with **15** and **18** in the presence of triethylamine (Scheme 3). The resulting fluorescent LO products **19** and **20** were subsequently purified by preparative-HPLC chromatography and were characterized by MS/MS (Figure 1 a,b) and NMR.

Characterization of 19 and 20

The tandem mass fragmentation pattern (Figure 1 a) and NMR spectra (Experimental Section) of **19** showed remarkable similarities to that of **4**.[25] However, a fragment loss of *m*/*z*: 451 in the MS/MS spectrum of **19** (Figure 1 a) instead of loss of *m*/*z*:



Scheme 2. Synthesis of amine derivative **18**: a) $NaBH_4$, I_2, THF, RT; g) ICH_2COOEt, DIPEA, NMP, 100 °C; h) *m*CPBA, DCM, RT; i) ethylenediamine, MeOH, reflux. NMP = *N*-methylpyrrolidone.



Scheme 3. Synthesis of fluorescent LOs **19** and **20**.

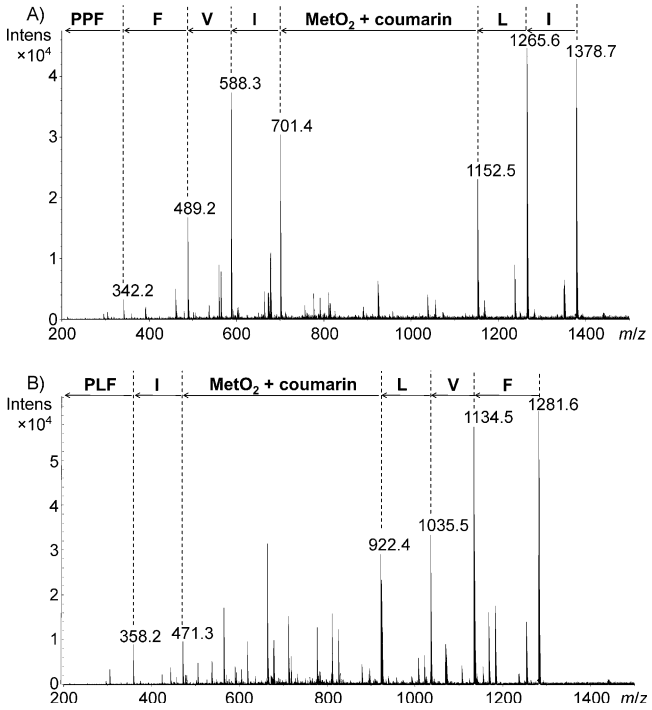


Figure 1. CID MS/MS spectrums of **19** (A) and **20** (B) showing the fragmentation pattern of the $MetO_2$ coumarin adduct.

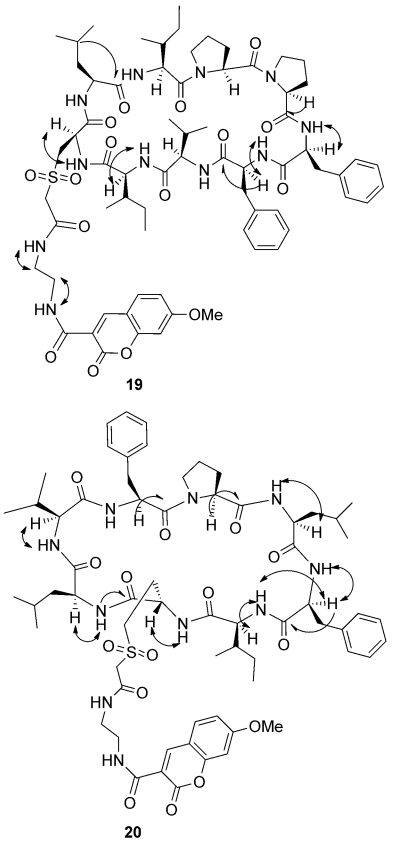


Figure 2. Structure of **19** (A) and **20** (B). Double arrows show selected NOE correlations. Half arrows show selected HMBC correlations.

163 ($MetO_2$) in **4** indicated the presence of a 7-methoxycoumarin-3-carboxylic acid conjugate of amine **15**. The protons attached to the heteroatoms were assigned from ^{1}H NMR, and their coupling to amide protons and carbonyl carbon atoms employed NOE and HMBC correlations (Figure 2 a,b). These provided the amino acid sequence of the fluorescent LOs. Sequential assignment of β, γ, and δ protons was carried out by using ^{1}H-^{1}H COSY and elucidation of attachments of these protons to carbon atoms was carried out by HMQC analysis. The amino acid sequence of **19** was further established using tandem mass spectrometry (Figure 1 a). Fluorescent LO **20** was similarly characterized. However, the ^{13}C NMR spectrum showed two sets of signals for each carbon atom corresponding to two conformers of **20** similar to **7**.[25] Signals from the major conformer are shown in the Experimental Section. Structures of **19** and **20** were confirmed by MS/MS and NMR. HPLC-MS analysis showed a $[M+H]^+$ ion peak at *m/z*: 1378.6751 corresponding to a molecular formula, $C_{70}H_{96}N_{11}O_{16}S$ for LO **19.** A *m/z*: 1281.6317 corresponded to the molecular formula, $C_{65}H_{89}N_{10}O_{15}S$ of **20**.

Fluorescence studies

The absorption and fluorescence studies were conducted in four different solvents (methanol, ethanol, acetonitrile, and DMF) at a concentration of 3×10^{-5} M. The samples were excited at 354 nm, and emission spectra were recorded. Coumarin 120 (7-amino-4-methyl-1, 2-benzopyrone) was used as a reference.[34]

7-Methoxycoumarin-3-carboxylic acid was used as a tag in which the methoxy group acts as an electron donor and carbonyl as an acceptor. The fluorescence intensity of this coumarin dye is solvent-dependent and it increases with solvent polarity with negligible emission shift.[35] It has been postulated that, nπ* and ππ* singlet states are close to each other in the

Table 2. Spectroscopic analysis of **19** in different solvents.

Solvent	λ max [nm]	λ emission [nm]	ϕ Quantum yield
ethanol	348	407	0.69
methanol	348	404	0.71
acetonitrile	345	402	0.57
DMF	347	408	0.44

Table 3. Spectroscopic analysis of **20** in different solvents.

Solvent	λ max [nm]	λ emission [nm]	ϕ Quantum yield
ethanol	348	403	0.62
methanol	347	403	0.68
acetonitrile	345	405	0.59
DMF	347	406	0.42

nonpolar solvent, in which intersystem crossing takes place and hence the fluorescence yield is lower. However, in polar solvent the difference between these two states is greater making intersystem crossing inefficient.[35b] Fluorescent LOs **19** and **20** absorb light at 347 nm and emit at 406 nm. Both show the same trend with higher quantum yields in methanol and ethanol than in either acetonitrile or DMF (Figures S37 and S38, Supporting Information; Tables 2 and 3). The hydrogen bonding of hydroxyl groups of alcohols has an extra effect when compared to aprotic acetonitrile and DMF. However, fluorescence in DMF occurs with very low quantum yield compared to fluorescence in other solvents. Potentially nonradiative decay or conformational changes occur in dyes that arise from interaction with the LO in DMF lowering quantum yield.

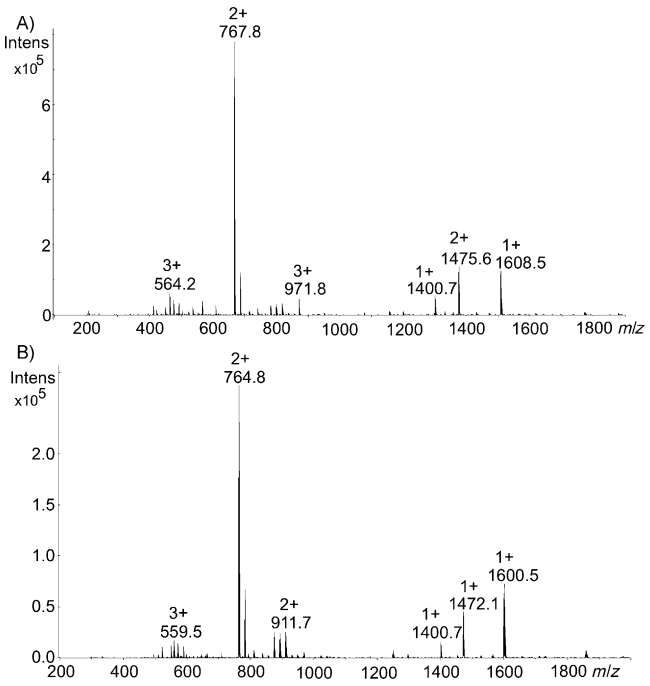
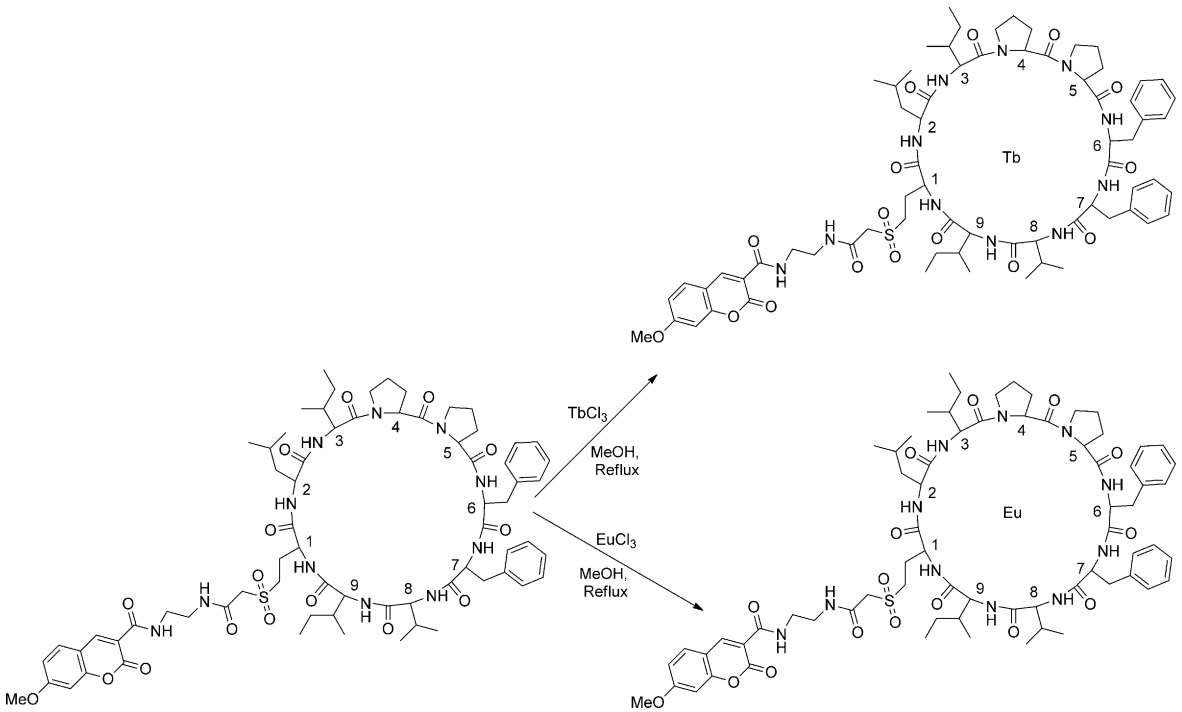


Figure 3. MS spectrums of **21** (A) and **22** (B).

Fluorescence energy transfer from coumaryl–LO to metals

LO **19** was mixed with solutions of metal ions ($TbCl_3$ and $EuCl_3$) to prepare LO metal adducts **21** (Tb) and **22** (Eu), respectively (Scheme 4). The resulting LO metal complexes **21** and **22** were characterized by MS (Figure 3 a,b). The potential fluorescence energy transfer from coumarin dye to a metal through LOs was investigated in different solvents. In this experiment, the



Scheme 4. Synthesis of LO metal complexes **21** (Tb) and **22** (Eu).

chromophore (coumarin) was excited with monochromatic light (354 nm).

In solvents (methanol, ethanol, and DMF) with exchangeable protons that participate in H-bonds and quenching no emissions or fluorescence (Tb, Eu bands) were observed. Eu complexes with LOs dissolved in acetonitrile in different concentrations produced a fluorescence emission spectrum (Eu band) that indicated possible fluorescence energy transfer from the fluorophore to Eu (Figure 4a). No similar emissions were observed for Tb complexes in acetonitrile. These results show that fluorescence energy transfer from coumarin to Eu occurs in acetonitrile (this is attributed to the lower solvation effects of acetonitrile).

The effects of Eu on coumarin quantum yield in acetonitrile were further investigated. As the number of equivalents of Eu increased, the quantum yield of LO–coumarin complexes decreased (Figure 4b). At higher ratios of Eu to LO–coumarin complex fluorescence energy is transferred between coumarin dye and Eu.

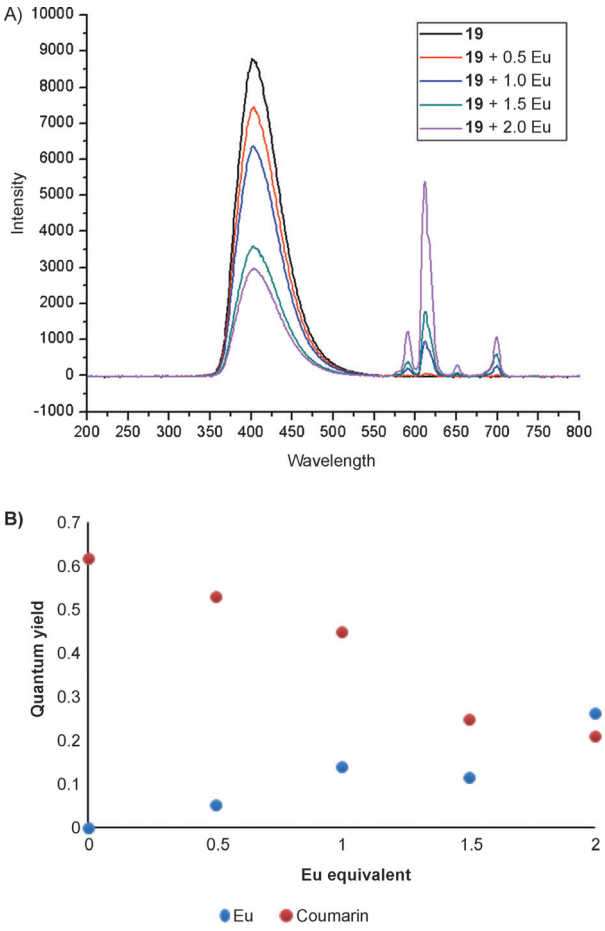


Figure 4. Fluorescence spectrum of **22** (A) and effect of Eu equivalents on the quantum yield of coumarin and Eu (B).

FRET utility of LOs

To determine FRET ability of LO **22** in biological environments, it was examined in living cells. A low concentration (476 nM) of LO **22** was administered to immortalized human dermal fibroblasts and incubated for 15 min. Cells were imaged by confocal fluorescence microscope by using 350 nm excitation (Figure 5). No fluorescence was seen in the cytoplasm of the control, whereas LO **22**-treated cells show red emission at 600 nm. Preliminary results show that LO **22** was accumulated in the cytoplasm and also confirms the ability of coumarin to excite Eu through the peptide.

LO metal adducts with coumarin might also have potential for use in organic light-emitting diodes (OLEDs). Previously, a LO analogue with tryptophan sensitizer was used to excite Tb^{3+} with an energy band gap of 2.9 eV. Charge transfer between LO and the complexed metal did not occur but the band gap was favourable to encourage future investigations.[36] Hence LO **22** with coumarin sensitizer might lower the band gap required for the construction of more efficient OLEDs.

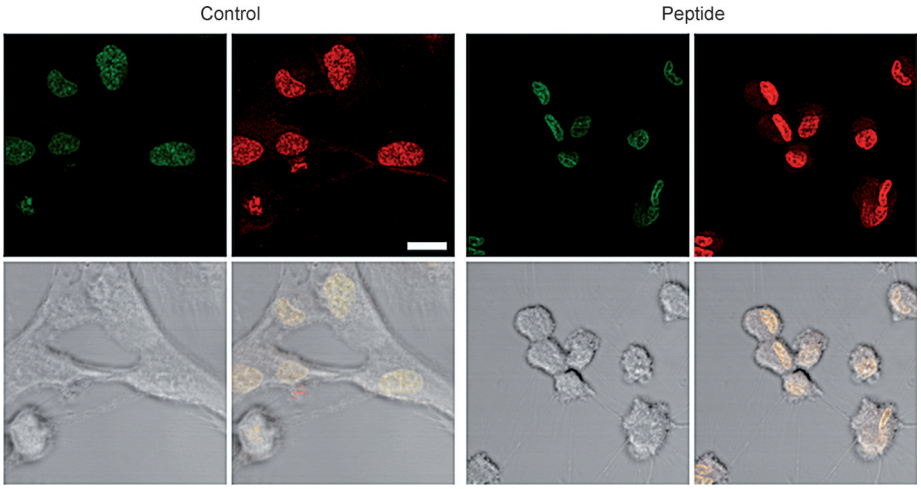


Figure 5. LO **22** localizes to the cytoplasm of human cells. NB1 hTERT immortalized human dermal fibroblasts grown on coverslips were either untreated (control) or cells (LO **22**) with 476 nM LO **22** for 15 min. Coverslips were then placed on 500 μL of cell culture media and live cells imaged. For both control and LO **22** treated conditions, cells were counterstained with the DNA specific dye Hoechst 33342 (green). Peptide localization is shown in red. For both control and LO **22** treated conditions a phase-contrast (grey scale) image was acquired and all channels merged to show localization. The red signal in the nucleus of control and peptide treated cells is due to the overlap in excitation of both the peptide and the Hoechst dye. Scale bar = 10 μm.

Conclusion

In summary, we have described a methodology for derivatization of LOs (**3** and **6**) using methionine as a point of attachment to give modified fluorescent LOs **19** and **20** that were utilized in fluorescence studies. We have also described methods for increas-

ing the stability of such compounds to heat, reduction, and oxidation. This strategy is suitable for making analogues of LOs for use in different applications. The structures of 20 derivatives (ester, nitrile, amine, and alcohol) were fully elucidated using HR HPLC-MS-ESI, tandem mass spectrometry, 1D (^{1}H and ^{13}C), and 2D NMR (NOE, COSY, HSQC and HMBC) spectroscopic methods. Fluorescence studies of coumarin complexes were conducted in different solvents and both compounds show high quantum yield in alcohols. This is the first report of their complete characterization. Eu complex **22** has shown fluorescence energy transfer from dye to metal. We also have successfully demonstrated FRET utility as a cytoplasm specific stain. These structures will probably provide a better understanding of the mechanism(s) of their biological activity and can possibly be used as fluorescent biosensors in biological research and drug discovery.

Experimental Section

General

All chemicals were purchased from Sigma–Aldrich and HPLC grade solvents were used. Compounds **3** and **6** were obtained from flax oil.[37] All other reagents were purchased from commercial sources and were used without further purification. The ^{1}H (500 MHz) and ^{13}C NMR (125.8 MHz) spectra were recorded on a Bruker Avance spectrometer (Bruker Biospin, Germany, Inverse triple resonance probe, TXI, 5 mm, XWIN-NMR 3.5 software). Spectra acquired at 320 K had improved resolution when compared to spectra obtained at lower temperatures (data not shown). Subsequent 2D NMR spectra were also acquired at 320 K. Proton and corresponding carbon signals were assigned using ^{1}H–^{1}H COSY and HMQC correlations. The correlations between amide protons, carbonyl carbons and α-CH protons were determined from NOE and HMBC experiments. ^{1}H NMR spectra were taken in $CDCl_3$ and referenced to appropriate solvent signals. Chemical shifts are reported in ppm units downfield from tetramethylsilane. FTIR spectra were recorded on a Bio-Rad FTS-40 spectrometer (Win-IR software, Version-4.14) using diffuse reflectance mode on samples dispersed in KBr (DRIFT); only diagnostic and/or intense peaks are reported.

HPLC-DAD analysis was performed with an Agilent 1200 series HPLC system equipped with a quaternary pump, autosampler, degasser and diode array detector (Agilent G1315C/D, 1024-element photodiode array, wavelength range 190–950 nm). The peaks were detected at wavelengths of 214 nm with a 10 nm bandwidth against a reference signal at 300 nm. Chromatography was conducted using a Chromolith SpeedRod RP-18e column (2 μm mesoporous size, 50×4.6 mm I.D.) equipped with an online filter (Chromolith RP-18e guard cartridge, 5×4.6 mm). The mobile phase consisted of H_2O/acetonitrile (70:30 to 30:70 in 4 min, 10:90 in 0.5 min, 70:30 in 0.5 min and equilibrated to 70:30 for 1 min) at a flow rate of 2 mL min^{-1}.[32] A Chromolith Semi*Prep* RP-18e column (2 μm particle size, 100×10 mm I.D.) in series with a matching guard column was used for preparative liquid chromatographic separations. The mobile phase consisted of H_2O/acetonitrile (80:20 for 0.5 min followed by a linear gradient to 10:90 in 4.5 min, 80:20 in 0.25 min and equilibrated to 80:20 for 0.75 min) at a flow rate of 5 mL min^{-1}.

Reverse-phase enrichment of LO reaction mixtures were also performed using Sep-Pak vac 20cc (5 g) tc_{18} cartridges (37–55 μm particle size, 125 Å, Waters, Ireland).

Mass spectra and MS/MS of **19** and **20** were acquired using a Micro-TOF-Q II Mass Spectrometer (Bruker Daltonik GmbH, Bremen, Germany) equipped with APCI source (Parameters: dry temp, 200 °C; Vaporizer temp, 450 °C; dry gas, 8 L min^{-1}; nebulizer; 1.6 bar; capillary voltage, 4000 V). Additional HR HPLC-QTOF MS analyses were performed with an Agilent 1100 HPLC connected to QSTAR XL spectrometer (Mass Spectrometer Hybrid Quadrupole-TOF LCMS/MS, Applied Biosystems, Toronto, Canada) with an electrospray ionization (ESI) source. Chromatographic separation was achieved at room temperature using a Hypersil ODS C-18 (Hewlett-Packward, Germany, 5 μm particle size silica, 100×2.1 mm) column. The mobile phase consisted of a linear gradient of solvent A, H_2O containing 0.1% formic acid, and solvent B, acetonitrile containing 0.1% formic acid. Elution was performed using a linear gradient from 55% of B to 90% of B within 25 min at a flow rate of 0.25 mL min^{-1}.

Fluorescence excitation and emission spectra were acquired with a π*-180 PiStar stopped-flow spectrometer (Applied Photophysics, Surrey, UK), both slit widths were set at 4 nm. The relative quantum yield of standard coumarin 120 (7-amino-4-methyl-1, 2-benzopyrone) in ethanol (0.98 in EtOH) was used to calculate quantum yield. Absorbance of all solutions between 200 and 700 nm was below 0.7 AU. Fluorescence spectra details: Intensity, 3 s, Scan time, 1, Boxcar width, 0, Range, 200–1000 nm. All solutions were excited at 354 nm.

Human dermal fibroblasts (NB1) immortalized with human telomerase (hTERT) as described previously,[38] were grown on coverslips in 5% CO_2 in DMEM media for 24 h at 37 °C. For LO treatments, 50 μL acetonitrile containing 10 μM LO **22** was added to 1 mL of media containing Hoescht 33342 (H33342; 1:500 dilution of 10 mg mL^{-1}) and added to the cells. Control and treated cells were then imaged using a Zeiss 710 LSM 2-photon confocal inverted microscope and Chameleon Coherent laser as a light source (Chameleon Vision laser system). For imaging, excitation wavelengths were set and 350 nm for H33342 dye and emission wavelengths acquired at 405 nm. For LO **22** imaging excitation was set at 350 nm and the emission wavelength set to 600 nm. Images were imported into Image J/Fiji imaging software (http://fiji.sc/Fiji) and processed using identical exposure times and contrast in both treated and control cells.

Synthesis

[Met]-LO2 (2): Sodium borohydride (1 equiv, 0.07 g) and iodine (1.5 equiv, 0.35 g) were added to a stirred solution of LO **3** (2 g, 1.9 mmol) in THF (25 mL). The resulting mixture was stirred at room temperature for 1 h and was subsequently treated with water and was extracted with CH_2Cl_2. The solvent was removed under reduced pressure using rotary evaporator and **2** was purified by flash column chromatography (1.8 g). ^{1}H NMR (500 MHz, $CDCl_3$, COSY, NOESY): Met^1: δ = 4.27 (1H, dd, 8.4, 8.2 Hz, α), 1.99 (2H, m, β), 2.57 (1H, m, γ), 2.49 (1H, m, γ), 2.07 (3H, s, εMe), 6.52 ppm (1H, d, 6.4 Hz, NH); Leu^2: δ = 3.65 (1H, m, α), 1.63 (2H, m, β), 1.99 (1H, m, γ), 0.94 (3H, d, 6.6 Hz, δMe), 0.92 ppm (3H, d, 6.6 Hz, δMe); Ile^3: δ = 4.44 (1H, m, α), 1.92 (1H, m, β), 1.78 (2H, m, γ), 1.05 (3H, d, 6.4 Hz, γMe), 0.84 (3H, t, 7.5 Hz, δMe), 7.34 ppm (1H, br s, NH); Pro^4: δ = 4.03 (1H, m, α), 2.24 (1H, m, β), 1.92 (1H, m, β), 2.10 (1H, m, γ), 1.99 (1H, m, γ), 4.19 (1H, m, δ), 3.72 (1H, m, δ); Pro^5: δ = 3.94 (1H, m, α), 1.92 (2H, m, β), 1.50 (1H, m, γ), 0.74 (1H, m, γ), 3.34 (1H, m, δ), 3.00 ppm (1H, m, δ); Phe^6: δ = 4.74 (1H, m, α), 3.35 (1H, m, β), 3.00 (1H, m, β), 7.26 (2H, m, δ), 7.18 (1H, m, ε), 7.10 (2H, m, ζ), 7.95 ppm (1H, m, NH); Phe^7: δ = 3.95 (1H, m, α), 3.21 (1H, m, β), 2.79 (1H, m, β),7.26 (2H, m, δ), 7.18 (1H, m, ε), 7.10 (2H,

m, ζ), 7.45 (1H, d, 6.4, NH); Val[8]: δ=3.89 (1H, m, α), 2.24 (1H, m, β), 1.05 (3H, d, 6.6 Hz, γMe), 1.00 (3H, d, 6.6 Hz, γMe), 8.09 ppm (1H, brs, NH); Ile[9]: δ=4.20 (1H, m, α), 1.99 (1H, m, β), 1.50 (2H, m, γ), 0.95 (3H, d, 6.6 Hz, γMe), 0.89 (3H, t, 7.5 Hz, δMe), 6.82 ppm (1H, br s, NH); ^{13}C NMR (125 MHz, $CDCl_3$, HMQC, HMBC): Met[1]: δ= 58.6 (α), 25.4 (β), 31.3 (γ), 15.3 (εMe), 170.7 ppm (C=O); Leu[2]: δ= 58.6 (α), 28.9 (β), 25.2 (γ), 23.4 (δMe), 21.4 (δMe), 171.4 (C=O); Ile[3]: δ=56.2 (α), 37.2 (β), 25.4 (γ), 16.1 (γMe), 11.0 (δMe), 171.5 ppm (C=O); Pro[4]: δ=60.9 (α), 31.5 (β), 21.5 (γ), 46.8 (δ), 170.0 ppm (C=O); Pro[5]: δ=54.8 (α), 37.5 (β), 24.7 (γ), 48.3 (δ), 174.7 ppm (C=O); Phe[6]: δ=60.9 (α), 36.0 (β), 137.2 (γ), 129.7 (δ), 129.0 (ε), 127.4 (ζ), 172.5 ppm (C=O); Phe[7]: δ=55.6 (α), 37.7 (β), 136.4 (γ), 129.1 (δ), 128.9 (ε), 126.8 (ζ), 175.0 ppm (C=O); Val[8]: δ=62.4 (α), 29.6 (β), 19.6 (γMe), 18.3 (γMe), 172.4 ppm (C=O); Ile[9]: δ=54.0 (α), 35.0 (β), 25.4 (γ), 15.4 (γMe), 10.8 (δMe), 171.5 ppm (C=O).

[Met]-LO1 (5): Similarly LO **6** (2 g, 2.0 mmol) was reduced with sodium borohydride (1 equiv, 0.08 g) and iodine (1.5 equiv, 0.39 g) to give **5** (1.8 g) using the procedure above for LO **2** (1.81 g). ^{1}H NMR (500 MHz, $CDCl_3$, COSY, NOESY): Met[1]: δ=4.69 (1H, m, α), 2.38 (1H, m, β), 1.83 (1H, m, β), 2.47 (2H, m, γ), 2.06 (3H, s, εMe), 2.11 (3H, s, εMe minor), 7.38 ppm (1H, m, NH); Leu[2]: δ=4.25 (1H, m, α), 2.27 (1H, m, β), 1.81 (1H, m, β), 1.92 (1H, m, γ), 0.90 (3H, m, δMe), 0.87 ppm (3H, m, δMe); Val[3]: δ=4.45 (1H, m, α), 2.07 (1H, m, β), 0.97 (3H, d, 6.4 Hz, γMe), 0.94 (3H, d, 6.4 Hz, γMe), 7.04 ppm (1H, d, 7.5, NH); Phe[4]: δ=4.29 (1H, m, α), 3.00 (2H, m, β), 7.39–7.17 (5H, m, δ, ε, ζ), 6.86 ppm (1H, brs, NH); Pro[5]: δ=4.31 (1H, m, α), 1.65 (1H, m, β), 1.52 (1H, m, β), 1.66 (1H, m, γ), 1.46 (1H, m, γ), 3.37 ppm (2H, m, δ); Leu[6]: δ=3.79 (1H, m, α), 2.21 (1H, m, β), 1.83 (1H, m, β), 1.57 (1H, m, γ), 0.94 (3H, m, δMe), 0.90 (3H, m, δMe), 7.59 ppm (1H, m, NH); Phe[7]: δ=4.32 (1H, m, α), 3.16 (1H, m, β), 3.02 (1H, m, β), 7.39–7.17 (5H, m, δ, ε, ζ), 7.57 ppm (1H, m, NH); Ile[8]: δ=4.09 (1H, m, α), 1.83 (1H, m, β), 1.57 (2H, m, γ), 0.81 (3H, d, 6.4 Hz, γMe), 0.87 (3H, m, δMe), 6.07 ppm (1H, brs, NH).

^{13}C NMR (125 MHz, $CDCl_3$, HMQC, HMBC): Met[1]: δ=52.4 (α), 29.8 (β), 29.9 (γ), 15.5 (εMe), 15.6 (εMe (minor)), 171.1 ppm (C=O); Leu[2]: δ=63.1 (α), 36.3 (β), 25.3 (γ), 23.4 (δMe), 22.1 (δMe), 172.1 ppm (C=O); Val[3]: δ=57.5 (α), 31.7 (β), 19.4 (γMe), 18.2 (γMe), 171.7 (C=O); Phe[4]: δ=51.3 (α), 38.8 (β), 135.4 (γ), 129.2 (δ), 129.2 (ε), 127.6 (ζ), 171.4 ppm (C=O); Pro[5]: δ=58.5 (α), 37.2 (β), 25.2 (γ), 46.5 (δ), 170.6 ppm (C=O); Leu[6]: δ=55.3 (α), 30.9 (β), 25.1 (γ), 23.3 (δMe), 21.4 (δMe), 173.1 ppm (C=O); Phe[7]: δ=53.4 (α), 37.4 (β), 135.8 (γ), 129.6 (δ), 129.5 (ε), 127.9 (ζ), 174.1 ppm (C=O); Ile[8]: δ=60.3 (α), 35.8 (β), 25.2 (γ), 16.3 (γMe), 11.7 (δMe), 172.3 ppm (C=O).

S$^{4.1}$-Cyanomethylhomocysteine LO2 (8): Compound **2** (50 mg, 0.05 mmol) was dissolved in *N*-methylpyrrolidone (2.5 mL) at room temperature. Diisopropylethylamine (2.5 equiv, 20 μL) and iodoacetonitrile (7 equiv, 25 μL) were sequentially added to the solution and the resulting mixture was heated to 100 °C for 20 h. The reaction mixture was extracted with EtOAc and combined organic extracts were washed with water and subsequently concentrated under high vacuum (3 mbar) to yield a crude mixture. This crude mixture was purified using Sep-Pak cartridges and analyzed by HPLC. LO nitrile **8** was obtained (0.027 g). ^{1}H NMR (500 MHz, $CDCl_3$, COSY, NOESY): Met[1]: δ=4.35 (1H, m, α), 2.41 (1H, m, β), 2.06 (1H, m, β), 2.86 (1H, m, γ), 2.78 (1H, m, γ), 3.28 (2H, s, SCH_2); Leu[2]: δ= 3.81 (1H, m, α), 1.62 (2H, m, β), 2.16 (1H, m, γ), 0.95 (3H, d, 6.4 Hz, δMe), 0.93 ppm (3H, d, 6.4 Hz, δMe); Ile[3]: δ=4.53 (1H, m, α), 1.92 (1H, m, β), 2.21 (2H, m, γ), 1.06 (3H, d, 6.8 Hz, γMe), 0.89 ppm (3H, t, 7.5, δMe); Pro[4]: δ=3.98 (1H, m, α), 2.22 (1H, m, β), 1.91 (1H, m, β), 2.03 (1H, m, γ), 1.89 (1H, m, γ), 4.11 (1H, m, δ), 3.74 ppm (1H, m, δ); Pro[5]: δ=4.02 (1H, m, α), 1.93 (2H, m, β), 1.51 (1H, m, γ), 0.87 (1H, m, γ), 3.34 (1H, m, δ), 3.06 ppm (1H, m, δ); Phe[6]: δ=4.72 (1H, m, α), 3.16 (1H, m, β), 2.77 (1H, m, β), 7.24 (3H, m, δ, ε), 7.07 (2H, m, ζ); Phe[7]: δ=4.23 (1H, m, α), 3.34 (2H, m, β), 7.30 (2H, m, δ), 7.24 (1H, m, ε), 7.15 (2H, m, ζ), 7.96 (1H, d, 5, NH); Val[8]: δ= 3.81 (1H, m, α), 2.19 (1H, m, β), 1.01 (3H, d, 7 Hz, γMe), 0.98 (3H, d, 7 Hz, γMe), 7.67 ppm (1H, brs, NH); Ile[9]: δ=4.29 (1H, m, α), 2.06 (1H, m, β), 1.65 (2H, m, γ), 0.95 (3H, d, 6.4 Hz, γMe), 0.87 (3H, t, 7.5 Hz, δMe), 6.51 ppm (1H, brs, NH); ^{13}C NMR (125 MHz, $CDCl_3$, HMQC, HMBC): Met[1]: δ=53.4 (α), 25.2 (β), 28.9 (γ), 17.1 (SCH_2), 116.7 (CN), 170.8 (C=O); Leu[2]: δ=62.7 (α), 29.6 (β), 25.4 (γ), 23.4 (δMe), 21.4 (δMe), 171.6 ppm (C=O); Ile[3]: δ=55.8 (α), 37.4 (β), 24.8 (γ), 15.3 (γMe), 11.0 (δMe), 172.0 (C=O); Pro[4]: δ=54.9 (α), 37.4 (β), 24.7 (γ), 48.3 (δ), 170.1 ppm (C=O); Pro[5]: δ=61.0 (α), 31.6 (β), 21.7 (γ), 47.0 (δ), 174.6 ppm (C=O); Phe[6]: δ=55.4 (α), 37.7 (β), 137.0 (γ), 129.6 (δ), 129.0 (ε), 127.4 (ζ), 172.2 ppm (C=O); Phe[7]: δ=58.7 (α), 35.8 (β), 136.4 (γ), 129.1 (δ), 128.9 (ε), 127.0 (ζ), 175.1 ppm (C=O); Val[8]: δ=58.6 (α), 29.6 (β), 19.5 (γMe), 18.4 (γMe), 172.2 ppm (C=O); Ile[9]: δ=58.7 (α), 35.3 (β), 24.8 (γ), 16.2 (γMe), 11.1 (δMe), 172.1 ppm (C=O).

1-2-Aminobutanoic acid LO2 (9): Compound **8** (30 mg, 0.03 mmol) was dissolved in dioxane: H_2O (3:1 v/v, 1.2 mL). Pd-C and LiOH were added to the reaction mixture that was stirred overnight at 140 °C under H_2 (1 atm). The reaction mixture was filtered then neutralized with a solution of sat. $NaHCO_3$. The reaction mixture was extracted with ethyl acetate then concentrated to obtain the crude product (**9**). HPLC-MS analyses indicated a $[M+H]^+$ ion at *m/z*: 1012.6275, corresponding to a molecular formula of $C_{55}H_{81}N_9O_9$.

S$^{4.1}$-Cyanomethylhomocysteine (*S*)-oxide LO3 (10): Compound **8** (30 mg, 0.03 mmol) was dissolved in THF/H_2O (5:2 v/v, 1.2 mL) and then cooled to 0 °C before addition of aqueous solution of $NaIO_4$ (0.8 mL, excess). The reaction mixture was stirred overnight and then filtered. After removal of THF under reduced pressure, EtOAc was added to the resulting aqueous solution, which was then washed successively with water and brine. The organic layer was evaporated to yield a (*S*)-oxide nitrile **10** (25 mg). HPLC-MS analyses indicated a $[M+H]^+$ ion at *m/z*: 1099.5958, corresponding to a molecular formula of $C_{57}H_{83}N_{10}O_{10}S$. The structure was further confirmed by LCMS/MS analyses.

S$^{4.1}$-Cyanomethylhomocysteine (*S*,*S*)-dioxide LO4 (11): Compound **8** (9.4 mg, 0.009 mmol) was dissolved in dichloromethane (1 mL) to which *m*CPBA (3 equiv, 6.4 mg) was added. The reaction mixture was stirred at room temperature for 1 h, and was sequentially washed with a solution of 10% $Na_2S_2O_3$ (5 mL), saturated aq. $NaHCO_3$, and H_2O to yield sulfonyl nitrile **11** (10 mg). ^{1}H NMR (500 MHz, $CDCl_3$, COSY, NOESY): $MetO_2$[1]: δ=4.85 (1H, m, α), 2.69 (1H, m, β), 2.35 (1H, m, β), 3.67 (1H, m, γ), 3.53 (1H, m, γ), 4.04 (2H, s, SO_2CH_2), 7.35 ppm (1H, m, NH); Leu[2]: δ=4.02 (1H, m, α), 2.12 (2H, m, β), 1.65 (1H, m, γ), 0.95 (3H, d, 6.4 Hz, δMe), 0.93 (3H, d, 6.4 Hz, δMe), 7.78 ppm (1H, brs, NH); Ile[3]: δ=4.48 (1H, m, α), 1.93 (1H, m, β), 1.70 (2H, m, γ), 1.11 (3H, d, 6.4 Hz, γMe), 0.87 (3H, m, δMe), 8.13 ppm (1H, brs, NH); Pro[4]: δ=3.87 (1H, m, α), 2.27 (1H, m, β), 1.70 (1H, m, β), 2.05 (1H, m, γ), 1.96 (1H, m, γ), 3.93 (1H, m, δ), 3.67 (1H, m, δ); Pro[5]: δ=4.12 (1H, m, α), 1.99 (2H, m, β), 1.47 (1H, m, γ), 1.24 (1H, m, γ), 3.35 ppm (2H, m, δ); Phe[6]: δ= 4.89 (1H, m, α), 3.04 (2H, dd, 4.2, 12.5 Hz, β), 7.26 (3H,m, δ, ε), 7.16 (2H, m, ζ), 7.19 ppm (1H, m, NH); Phe[7]: δ=4.07 (1H, m, α), 3.39 (2H, m, β), 7.34 (2H, m, δ), 7.26 (1H, m, ε), 7.16 (2H, m, ζ), 7.61 ppm (1H, brs, NH); Val[8]: δ=3.87 (1H, m, α), 2.12 (1H, m, β), 0.95 (3H, m, γMe), 0.94 (3H, m, γMe), 6.47 ppm (1H, brs, NH); Ile[9]: δ=4.58 (1H, m, α), 1.99 (1H, m, β), 2.16 (2H, m, γ), 0.97 (3H, m, γMe), 0.84 (3H, m, δMe), 6.73 ppm (1H, brs, NH); ^{13}C NMR (125 MHz, $CDCl_3$, HMQC, HMBC): $MetO_2$[1]: δ=51.7 (α), 25.4 (β), 50.8 (γ), 42.5 (SO_2CH_2), 110.2 (CN), 171.1 ppm (C=O); Leu[2]: δ=61.0 (α), 38.4 (β), 25.4 (γ), 22.9 (δMe), 21.9 (δMe), 171.1 (C=O); Ile[3]: δ=56.3

(α), 36.6 (β), 24.8 (γ), 15.6 (γMe), 11.2 (δMe), 171.8 (C=O); Pro[4]: δ = 61.8 (α), 28.5 (β), 25.1 (γ), 48.1 (δ), 170.4 ppm (C=O); Pro[5]: δ = 61.0 (α), 32.1 (β), 21.9 (γ), 47.4 (δ), 173.8 ppm (C=O); Phe[6]: δ = 53.4 (α), 35.1 (β), 136.1 (γ), 129.7 (δ), 128.9 (ε), 127.5 (ζ), 173.8 ppm (C=O); Phe[7]: δ = 57.8 (α), 36.7 (β), 135.4 (γ), 129.1 (δ), 128.9 (ε), 127.3 (ζ), 175.1 ppm (C=O); Val[8]: δ = 59.1 (α), 29.3 (β), 19.6 (γMe), 18.2 (γMe), 173.0 (C=O); Ile[9]: δ = 58.3 (α), 36.8 (β), 24.8 (γ), 16.1 (γMe), 11.3 (δMe), 171.8 ppm (C=O).

$S^{4.1}$-(2-Aminoethyl)homocysteine (*S*,*S*)-dioxide LO4 (12): Compound **11** (8 mg, 0.007 mmol) was dissolved in acetic acid (0.5 mL) under argon and PtO_2 was added. The reaction mixture was stirred at rt under H_2 (1 atm) overnight. The reaction mixture was filtered and then neutralized with sat. $NaHCO_3$. The reaction mixture was extracted with ethyl acetate then concentrated to give the crude product (**12**). HPLC-MS analyses indicated a $[M+H]^+$ ion at *m/z*: 1119.6275, corresponds to a molecular formula of $C_{57}H_{83}N_{10}O_{10}S$.

$S^{4.1}$-Ethoxycarbonylmethylhomocysteine LO2 (13): Compound **2** (0.3 g, 0.28 mmol) was dissolved in *N*-methylpyrrolidone (10 mL) at room temperature. Diisopropylethylamine (2.5 equiv, 0.12 mL) and iodoacetate (7 equiv, 0.23 mL) were sequentially added to the solution and the resulting mixture was heated to 100 °C with stirring for 20 h. The reaction mixture was extracted with EtOAc and the combined organic extracts were washed with water and subsequently concentrated under high vacuum to yield a crude mixture that was purified using Sep-Pak cartridges and analyzed by HPLC. The ester (–COOEt) derivative **13** was obtained (0.27 g). ^{1}H NMR (500 MHz, $CDCl_3$, COSY, NOESY): Met[1]: δ = 4.27 (1H, dd, 8, 8.4 Hz, α), 1.95 (2H, m, β), 2.63 (1H, m, γ), 2.44 (1H, m, γ), 3.19 (2H, s, SCH_2), 4.16 (2H, q, 7 Hz, SCH_2COOCH_2), 1.26 (3H, t, 7 Hz, $SCH_2COOCH_2CH_3$), 6.44 ppm (1H, brs, NH); Leu[2]: δ = 3.61 (1H, m, α), 2.17 (2H, m, β), 1.98 (1H, m, γ), 0.93 (3H, d, 7 Hz, δMe), 0.91 (3H, d, 7 Hz, δMe); Ile[3]: δ = 4.42 (1H, m, α), 1.91 (1H, m, β), 2.22 (2H, m, γ), 1.00 (3H, d, 6.6 Hz, γMe), 0.83 (3H, t, 7 Hz, δMe), 7.44 ppm (1H, brs, NH); Pro[4]: δ = 4.03 (1H, brs, α), 2.22 (1H, m, β), 1.91 (1H, m, β), 2.02 (1H, m, γ), 1.87 (1H, m, γ), 4.16 (1H, brs, δ), 3.71 (1H, m, δ); Pro[5]: δ = 3.95 (1H, m, α), 1.95 (2H, m, β), 1.52 (1H, m, γ), 0.85 (1H, m, γ), 3.33 (1H, m, δ), 2.98 ppm (1H, m, δ); Phe[6]: δ = 4.71 (1H, m, α), 3.21 (1H, m, β), 2.77 (1H, m, β), 7.25 (2H, m, δ), 7.19 (1H, m, ε), 7.09 (2H, m, ζ) 7.45 ppm (1H, brs, NH); Phe[7]: δ = 4.03 (1H, m, α), 3.36 (2H, m, β), 7.25 (2H, m, δ), 7.19 (1H, m, ε), 7.09 (2H, m, ζ);; Val[8]: δ = 3.87 (1H, m, α), 2.22 (1H, m, β), 1.05 (3H, d, 7.3 Hz, γMe), 1.03 (3H, d, 7.3 Hz, γMe), 8.09 ppm (1H, brs, NH); Ile[9]: δ = 4.16 (1H, m, α), 2.10 (1H, m, β), 1.61 (2H, m, γ), 0.94 (3H, d, 7 Hz, γMe), 0.87 (3H, t, 7 Hz, δMe), 6.79 ppm (1H, brs, NH).

^{13}C NMR (125 MHz, $CDCl_3$, HMQC, HMBC): Met[1]: δ = 58.1 (α), 25.4 (β), 29.8 (γ), 33.7 (SCH_2), 60.9 (SCH_2COOCH_2), 14.3 ($SCH_2COOCH_2CH_3$), 169.8 ($COOCH_2CH_3$), 170.7 ppm (C=O); Leu[2]: δ = 58.5 (α), 28.8 (β), 25.1 (γ), 23.5 (δMe), 21.3 (δMe), 171.2 ppm (C=O); Ile[3]: δ = 56.1 (α), 37.1 (β), 25.3 (γ), 16.1 (γMe), 11.1 (δMe), 171.5 (C=O); Pro[4]: δ = 54.6 (α), 37.4 (β), 24.7 (γ), 48.3 (δ), 170.6 ppm (C=O); Pro[5]: δ = 61.5 (α), 31.5 (β), 21.5 (γ), 46.8 (δ), 174.8 ppm (C=O); Phe[6]: δ = 55.7 (α), 37.7 (β), 137.2 (γ), 129.6 (δ), 128.9 (ε), 127.4 (ζ), 172.4 ppm (C=O); Phe[7]: δ = 61.5 (α), 35.7 (β), 136.4 (γ), 129.0 (δ), 128.8 (ε), 126.7 (ζ), 175.1 ppm (C=O); Val[8]: δ = 62.4 (α), 29.5 (β), 19.6 (γMe), 18.3 (γMe), 172.4 ppm (C=O); Ile[9]: δ = 53.7 (α), 34.9 (β), 25.3 (γ), 15.2 (γMe), 10.7 (δMe), 172.0 ppm (C=O).

$S^{4.1}$-Ethoxycarbonylmethylhomocysteine LO1 (16): Similarly **5** (1 g, 1.0 mmol) was activated with iodoacetate (7 equiv, 1.55 g, 0.21 mL) and diisopropylethylamine (2.5 equiv, 0.36 g, 0.45 mL) in *N*-methylpyrrolidone (8 mL) using the above procedure to give **16** (1.1 g). ^{1}H NMR (500 MHz, $CDCl_3$, COSY, NOESY): Met[1]: δ = 4.69 (1H, m, α), 2.45 (1H, m, β), 1.84 (1H, m, β), 2.62 (2H, m, γ), 3.19 (2H, s, SCH_2), 4.14 (2H, q, 7 Hz, SCH_2COOCH_2), 1.25 (3H, t, 7 Hz, $SCH_2COOCH_2CH_3$), 7.42 ppm (1H, d, 9, NH); Leu[2]: δ = 4.31 (1H, m, α), 2.30 (1H, m, β), 1.75 (1H, m, β), 1.95 (1H, m, γ), 0.86 ppm (6H, d, 6.6 Hz, δMe); Val[3]: δ = 4.46 (1H, m, α), 2.06 (1H, m, β), 0.96 (3H, d, 6.4 Hz, γMe), 0.94 (3H, d, 6.4 Hz, γMe), 7.03 ppm (1H, d, 8.6, NH); Phe[4]: δ = 4.32 (1H, m, α), 3.01 (2H, m, β), 7.38–7.15 (5H, m, δ, ε, ζ), 6.72 (1H, brs, NH); Pro[5]: δ = 4.31 (1H, m, α), 1.57 (1H, m, β), 1.45 (1H, m, β), 1.64 (1H, m, γ), 1.50 (1H, m, γ), 3.37 ppm (2H, m, δ); Leu[6]: δ = 3.79 (1H, m, α), 2.22 (1H, m, β), 1.84 (1H, m, β), 1.57 (1H, m, γ), 0.87 (6H, m, δMe), 7.53 ppm (1H, d, 7 Hz, NH); Phe[7]: δ = 4.30 (1H, m, α), 3.16 (1H, m, β), 3.04 (1H, m, β), 7.38–7.15 (5H, m, δ, ε, ζ), 7.59 ppm (1H, brs, NH); Ile[8]: δ = 4.11 (1H, m, α), 1.84 (1H, m, β), 1.57 (2H, m, γ), 0.80 (3H, d, 6.4 Hz, γMe), 0.87 (3H, m, δMe), 6.08 ppm (1H, brs, NH); ^{13}C NMR (125 MHz, $CDCl_3$, HMQC, HMBC): Met[1]: δ = 52.2 (α), 31.1 (β), 29.6 (γ), 33.8 (SCH_2), 61.4 (SCH_2COOCH_2), 14.3 ($SCH_2COOCH_2CH_3$), 170.5 (S-$CH_2COOCH_2CH_3$), 171.1 (C=O); Leu[2]: δ = 51.3 (α), 29.9 (β), 30.8 (γ), 23.2 (δMe), 22.1 (δMe), 173.1 ppm (C=O); Val[3]: δ = 57.4 (α), 31.6 (β), 19.4 (γMe), 18.1 (γMe), 171.7 ppm (C=O); Phe[4]: δ = 53.3 (α), 38.8 (β), 135.4 (γ), 129.2 (δ), 129.1 (ε), 127.6 (ζ), 171.3 (C=O); Pro[5]: δ = 63.2 (α), 38.2 (β), 25.3 (γ), 46.5 (δ), 170.8 ppm (C=O); Leu[6]: δ = 55.3 (α), 37.2 (β), 25.1 (γ), 22.8 (δMe), 21.9 (δMe), 173.1 ppm (C=O); Phe[7]: δ = 58.4 (α), 37.4 (β), 135.9 (γ), 129.6 (δ), 129.5 (ε), 127.9 (ζ), 174.1 ppm (C=O); Ile[8]: δ = 60.2 (α), 35.8 (β), 25.3 (γ), 16.3 (γMe), 11.7 (δMe), 172.3 ppm (C=O).

$S^{4.1}$-Ethoxycarbonylmethylhomocysteine (*S*,*S*)-dioxide LO4 (14): Compound **13** (0.15 g, 0.13 mmol) was dissolved in dichloromethane (5 mL) to which *m*CPBA (3 equiv, 0.1 g) was added. The reaction mixture was stirred at room temperature for 1 h, and then sequentially washed with solutions of 10% $Na_2S_2O_3$ (5 mL), saturated aq. $NaHCO_3$, and H_2O to yield methionine (*S*,*S*)-dioxide **14** (0.16 g). ^{1}H NMR (500 MHz, $CDCl_3$, COSY, NOESY): $MetO_2$[1]: δ = 4.61 (1H, m, α), 2.46 (2H, m, β), 3.46 (2H, m, γ), 3.95 (2H, s, SO_2CH_2), 4.23 (2H, q, 7 Hz, $SO_2CH_2COOCH_2$), 1.29 (3H, t, 7 Hz, $SO_2CH_2COOCH_2CH_3$), 7.20 ppm (1H, d, 7, NH); Leu[2]: δ = 4.06 (1H, d, 7.9 Hz, α), 2.16 (2H, m, β), 1.63 (1H, m, γ), 0.95 (3H, d, 6.4 Hz, δMe), 0.93 (3H, d, 6.4 Hz, δMe), 7.63 ppm (1H, d, 6 Hz, NH); Ile[3]: δ = 4.48 (1H, m, α), 1.93 (1H, m, β), 1.50 (2H, m, γ), 1.07 (3H, d, 6.8 Hz, γMe), 0.84 (3H, t, 6.8 Hz, δMe), 6.60 (1H, brs, NH); Pro[4]: δ = 3.81 (1H, m, α), 2.16 (1H, m, β), 1.67 (1H, m, β), 2.17 (1H, m, γ), 1.95 (1H, m, γ), 4.01 (1H, m, δ), 3.46 ppm (1H, m, δ); Pro[5]: δ = 4.06 (1H, m, α), 2.16 (1H, m, β), 1.94 (1H, m, β), 1.49 (1H, m, γ), 0.96 (1H, m, γ), 3.29 ppm (2H, m, δ); Phe[6]: δ = 4.85 (1H, m, α), 3.09 (2H, dd, 4.2, 12.5 Hz, β), 7.24 (2H, m, δ), 7.20 (1H, m, ε), 7.12 (2H, m, ζ); Phe[7]: δ = 3.99 (1H, m, α), 3.67 (2H, m, β), 7.30 (3H, m, δ, ε), 7.12 (2H, m, ζ) 7.72 ppm (1H, brs, NH); Val[8]: δ = 3.87 (1H, m, α), 2.16 (1H, m, β), 0.95 (3H, d, 6.4 Hz, γMe), 0.93 (3H, d, 6.4 Hz, γMe); Ile[9]: δ = 4.48 (1H, m, α), 1.95 (1H, m, β), 2.16 (2H, m, γ), 0.92 (3H, d, 6.4 Hz, γMe), 0.90 ppm (3H, t, 6.4 Hz, δMe); ^{13}C NMR (125 MHz, $CDCl_3$, HMQC, HMBC): $MetO_2$[1]: δ = 52.4 (α), 25.3 (β), 51.2 (γ), 57.6 (SO_2CH_2), 62.7 ($SO_2CH_2COOCH_2$), 14.1 ($SO_2CH_2COOCH_2CH_3$), 162.8 ($COOCH_2CH_3$), 170.6 ppm (C=O); Leu[2]: δ = 61.0 (α), 37.9 (β), 25.4 (γ), 23.2 (δMe), 21.7 (δMe), 171.4 (C=O); Ile[3]: δ = 56.3 (α), 36.2 (β), 24.8 (γ), 15.6 (γMe), 11.1 (δMe), 171.7 ppm (C=O); Pro[4]: δ = 62.0 (α), 28.6 (β), 25.1 (γ), 48.1 (δ), 170.5 ppm (C=O); Pro[5]: δ = 56.6 (α), 31.9 (β), 21.7 (γ), 47.1 (δ), 174.1 ppm (C=O); Phe[6]: δ = 54.2 (α), 36.1 (β), 136.4 (γ), 129.8 (δ), 129.0 (ε), 127.4 (ζ), 173.0 ppm (C=O); Phe[7]: δ = 58.3 (α), 36.2 (β), 136.3 (γ), 128.9 (δ), 127.3 (ε), 127.3 (ζ), 175.2 ppm (C=O); Val[8]: δ = 58.8 (α), 29.4 (β), 19.6 (γMe), 18.3 (γMe), 172.8 ppm (C=O); Ile[9]: δ = 58.3 (α), 36.7 (β), 25.0 (γ), 16.1 (γMe), 11.3 (δMe), 171.8 ppm (C=O).

$S^{4.1}$-Ethoxycarbonylmethylhomocysteine (*S*,*S*)-dioxide LO7 (17): Similarly **16** (0.7 g, 0.68 mmol) was oxidized with *m*CPBA (3 equiv, 0.35 g) in dichloromethane (15 mL) using the above procedure to

give **17** (0.74 g). ^{1}H NMR (500 MHz, $CDCl_3$, COSY, NOESY): $MetO_2^1$: $\delta = 4.71$ (1H, m, α), 2.58 (1H, m, β), 2.18 (1H, m, β), 3.52 (1H, m, γ), 3.17 (1H, m, γ), 3.92 (2H, dd, 14.6, 14.6, SO_2CH_2), 4.23 (2H, q, 7 Hz, $SO_2CH_2COOCH_2$), 1.29 (3H, t, 7 Hz, $SO_2CH_2COOCH_2CH_3$), 7.69 ppm (1H, brs, NH); Leu^2: $\delta = 4.33$ (1H, m, α), 2.32 (1H, m, β), 1.72 (1H, m, β), 1.95 (1H, m, γ), 0.88 (3H, m, δMe), 0.81 (3H, m, δMe), 6.83 (1H, brs, NH); Val^3: $\delta = 4.47$ (1H, m, α), 2.05 (1H, m, β), 0.97 (3H, d, 6.8 Hz, γMe), 0.94 (3H, d, 6.8 Hz, γMe), 6.97 ppm (1H, brs, NH); Phe^4: $\delta = 4.34$ (1H, m, α), 3.02 (2H, m, β), 7.39–7.18 (5H, m, δ, ε, ζ), 6.66 ppm (1H, brs, NH); Pro^5: $\delta = 4.12$ (1H, m, α), 1.72 (1H, m, β), 1.42 (1H, m, β), 1.65 (1H, m, γ), 1.47 (1H, m, γ), 3.36 ppm (2H, m, δ); Leu^6: $\delta = 3.79$ (1H, m, α), 2.18 (1H, m, β), 1.86 (1H, m, β), 1.54 (1H, m, γ), 0.94 (3H, d, 6.8 Hz, δMe), 0.88 (3H, m, δMe), 7.61 ppm (1H, d, 7 Hz, NH); Phe^7: $\delta = 4.31$ (1H, m, α), 3.17 (1H, m, β), 3.07 (1H, m, β), 7.39–7.18 (5H, m, δ, ε, ζ), 7.69 ppm (1H, brs, NH); Ile^8: $\delta = 4.07$ (1H, m, α), 1.86 (1H, m, β), 1.54 (2H, m, γ), 0.79 (3H, m, γMe), 0.86 (3H, m, δMe), 6.12 ppm (1H, brs, NH); ^{13}C NMR (125 MHz, $CDCl_3$, HMQC, HMBC): $MetO_2^1$: $\delta = 51.3$ (α), 24.8 (β), 51.4 (γ), 57.5 (SO_2CH_2), 62.7 ($SO_2CH_2COOCH_2$), 14.1 ($SO_2CH_2COOCH_2CH_3$), 162.9 ($SO_2CH_2COOCH_2CH_3$), 170.9 ppm (C=O); Leu^2: $\delta = 51.2$ (α), 37.0 (β), 25.4 (γ), 23.3 (δMe), 21.8 (δMe), 171.6 (C=O); Val^3: $\delta = 57.5$ (α), 32.0 (β), 19.4 (γMe), 18.2 (γMe), 171.6 ppm (C=O); Phe^4: $\delta = 53.1$ (α), 38.9 (β), 135.5 (γ), 129.2 (δ), 129.1 (ε), 127.6 (ζ), 171.2 ppm (C=O); Pro^5: $\delta = 54.2$ (α), 31.0 (β), 25.2 (γ), 46.5 (δ), 170.4 ppm (C=O); Leu^6: $\delta = 55.3$ (α), 29.9 (β), 29.3 (γ), 23.0 (δMe), 21.4 (δMe), 173.4 ppm (C=O); Phe^7: $\delta = 58.4$ (α), 37.3 (β), 135.6 (γ), 129.6 (δ), 129.5 (ε), 127.9 (ζ), 174.6 ppm (C=O); Ile^8: $\delta = 60.2$ (α), 35.7 (β), 25.0 (γ), 16.3 (γMe), 11.7 (δMe), 172.5 ppm (C=O).

$S^{4.1}$-[2-(2-Aminoethylamino)-2-oxo-ethyl]homocysteine (*S*,*S*)-dioxide LO4 (15): Compound **14** (0.1 g, 0.09 mmol) was dissolved in methanol and ethylene diamine (excess) was added. The resulting reaction mixture was refluxed overnight. The organic solvents were evaporated and the resulting crude product was purified using Sep-Pak cartridges and analyzed by HPLC. LO **15** was obtained (0.09 g). ^{1}H NMR (500 MHz, $CDCl_3$, COSY, NOESY): $MetO_2^1$: $\delta = 4.72$ (1H, m, α), 2.50 (1H, m, β), 2.40 (1H, m, β), 3.48 (1H, m, γ), 3.41 (1H, m, γ), 3.90 (2H, s, SO_2CH_2), 2.87 (4H, m, $NHCH_2CH_2NH_2$), 7.41 ppm (1H, brs, NH); Leu^2: $\delta = 3.88$ (1H, brs, α), 2.17 (2H, brs, β), 1.64 (1H, m, γ), 0.92 (6H, d, 6.6 Hz, δMe), 7.75 ppm (1H, brs, NH); Ile^3: $\delta = 4.47$ (1H, m, α), 1.92 (1H, m, β), 1.50 (2H, m, γ), 1.08 (3H, d, 6.6 Hz, γMe), 0.86 (3H, t, 7 Hz, δMe), 8.03 ppm (1H, brs, NH); Pro^4: $\delta = 3.79$ (1H, brs, α), 2.17 (1H, m, β), 1.64 (1H, m, β), 2.04 (1H, m, γ), 1.94 (1H, m, γ), 3.97 (1H, brs, δ), 3.65 ppm (1H, m, δ); Pro^5: $\delta = 4.11$ (1H, m, α), 2.12 (1H, m, β), 1.95 (1H, m, β), 1.50 (1H, m, γ), 0.96 (1H, m, γ), 3.31 ppm (2H, m, δ); Phe^6: $\delta = 4.86$ (1H, m, α), 3.10 (1H, m, β), 3.00 (1H, m, β), 7.24 (3H, m, δ, ε), 7.15 ppm (2H, m, ζ); Phe^7: $\delta = 3.97$ (1H, m, α), 3.31 (2H, m, β), 7.32 (2H, m, δ), 7.24 (1H, m, ε), 7.15 (2H, m, ζ) 7.67 ppm (1H, brs, NH); Val^8: $\delta = 3.88$ (1H, m, α), 2.17 (1H, m, β), 0.92 ppm (6H, d, 6.6 Hz, γMe); Ile^9: $\delta = 4.53$ (1H, m, α), 1.98 (1H, m, β), 1.64 (2H, m, γ), 0.96 (3H, d, 6.4 Hz, γMe), 0.84 (3H, t, 6.4 Hz, δMe), 6.78 ppm (1H, brs, NH); ^{13}C NMR (125 MHz, $CDCl_3$, HMQC, HMBC): $MetO_2^1$: $\delta = 57.2$ (α), 25.1 (β), 50.7 (γ), 58.6 (SO_2CH_2), 42.9 ($NHCH_2CH_2NH_2$), 41.2 ($NHCH_2CH_2NH_2$), 171.9 ppm (C=O); Leu^2: $\delta = 61.8$ (α), 29.9 (β), 25.5 (γ), 23.1 (δMe), 21.9 (δMe), 171.9 (C=O); Ile^3: $\delta = 56.3$ (α), 36.7 (β), 25.4 (γ), 15.6 (γMe), 11.2 (δMe), 171.9 ppm (C=O); Pro^4: $\delta = 59.1$ (α), 28.7 (β), 25.1 (γ), 48.2 (δ), 170.9 ppm (C=O); Pro^5: $\delta = 61.2$ (α), 32.0 (β), 21.7 (γ), 47.3 (δ), 173.6 ppm (C=O); Phe^6: $\delta = 54.2$ (α), 35.6 (β), 136.4 (γ), 129.8 (δ), 129.0 (ε), 127.4 (ζ), 173.6 ppm (C=O); Phe^7: $\delta = 61.2$ (α), 38.4 (β), 135.9 (γ), 129.1 (δ), 128.9 (ε), 127.3 (ζ), 175.2 ppm (C=O); Val^8: $\delta = 59.1$ (α), 29.4 (β), 19.7 (γMe), 18.3 (γMe), 173.2 ppm (C=O); Ile^9: $\delta = 58.3$ (α), 36.7 (β), 25.4 (γ), 16.1 (γMe), 11.3 (δMe), 173.0 ppm (C=O).

$S^{4.1}$-[2-(2-Aminoethylamino)-2-oxo-ethyl]homocysteine (*S*,*S*)-dioxide LO7 (18): Similarly **17** (0.3 g, 0.29 mmol) was coupled with ethylene diamine (excess) in methanol (6 mL) to give **18** (0.27 g) using the procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$, COSY, NOESY) $MetO_2^1$: $\delta = 4.92$ (1H, m, α), 2.58 (1H, m, β), 2.20 (1H, m, β), 3.43 (2H, m, γ), 3.41 (2H, m, SO_2CH_2), 3.06 (2H, m, $NHCH_2CH_2NH_2$), 3.43 (2H, m, $NHCH_2CH_2$ NH_2), 4.36 (2H, brs, $NHCH_2CH_2NH_2$), 6.81 ppm (1H, m, NH); Leu^2: $\delta = 4.01$ (1H, m, α), 1.74 (1H, m, β), 1.65 (1H, m, β), 2.38 (1H, m, γ), 0.84 (3H, d, 6.6 Hz, δMe), 0.78 (3H, d, 6.6 Hz, δMe), 8.97 ppm (1H, brs, NH); Val^3: $\delta = 4.19$ (1H, m, α), 2.38 (1H, m, β), 0.95 (6H, m, γMe), 6.49 ppm (1H, brs, NH); Phe^4: $\delta = 4.81$ (1H, m, α), 3.22 (1H, m, β), 3.12 (1H, m, β), 7.25–7.06 (5H, m, δ, ε, ζ), 7.02 ppm (1H, m, NH); Pro^5: $\delta = 4.47$ (1H, m, α), 2.38 (1H, m, β), 1.75 (1H, m, β), 2.04 (1H, m, γ), 1.95 (1H, m, γ), 3.73 (1H, m, δ), 3.52 ppm (1H, m, δ); Leu^6: $\delta = 3.45$ (1H, m, α), 2.05 (1H, m, β), 1.95 (1H, m, β), 1.75 (1H, m, γ), 0.92 (6H, m, δMe), 8.11 ppm (1H, m, NH); Phe^7: $\delta = 5.24$ (1H, m, α), 3.07 (2H, m, β), 7.25–7.06 (5H, m, δ, ε, ζ), 7.52 ppm (1H, brs, NH); Ile^8: $\delta = 4.08$ (1H, m, α), 1.28 (1H, m, β), 1.46 (1H, m, γ), 1.41 (1H, m, γ), 1.04 (3H, m, γMe), 0.91 ppm (3H, m, δMe); ^{13}C NMR (125 MHz, $CDCl_3$, HMQC, HMBC): $MetO_2^1$: $\delta = 50.3$ (α), 25.0 (β), 50.8 (γ), 60.4 (SO_2CH_2), 40.4 ($NHCH_2CH_2NH_2$), 40.7 ($NHCH_2CH_2NH_2$), 163.1 ($CONHCH_2CH_2NH_2$), 170.5 ppm (C=O); Leu^2: $\delta = 56.4$ (α), 39.8 (β), 29.6 (γ), 23.0 (δMe), 22.3 (δMe), 172.9 ppm (C=O); Val^3: $\delta = 59.7$ (α), 30.5 (β), 19.6 (γMe), 17.6 (γMe), 171.8 ppm (C=O); Phe^4: $\delta = 54.5$ (α), 36.7 (β), 138.0 (γ), 129.0 (δ), 128.7 (ε), 126.7 (ζ), 172.9 ppm (C=O); Pro^5: $\delta = 63.7$ (α), 29.8 (β), 25.0 (γ), 48.4 (δ), 173.9 ppm (C=O); Leu^6: $\delta = 59.7$ (α), 25.6 (β), 25.0 (γ), 22.5 (δMe), 22.0 (δMe), 173.4 ppm (C=O); Phe^7: $\delta = 50.8$ (α), 35.9 (β), 137.3 (γ), 128.8 (δ), 128.3 (ε), 126.6 (ζ), 172.3 ppm (C=O); Ile^8: $\delta = 54.5$ (α), 25.3 (β), 39.8 (γ), 17.6 (γMe), 10.2 (δMe), 172.3 ppm (C=O).

7-Methoxycoumarin-3-carboxylic succinimidyl ester: 7-Methoxycoumarin-3-carboxylic succinimidyl ester was prepared as previously described.[33] 7-Methoxycoumarin-3-carboxylic acid (0.22 g, 1 mmole) was dissolved in DMF (2.5 mL) and *N*-hydroxy succinimide ester (1 equiv, 0.12 g) was added. The reaction mixture was cooled to 0 °C, DCC (1.1 equiv, 0.23 g) was added and the mixture was stirred for 30 min at 0 °C and then warmed to RT and stirred for an additional 2 h. The solution was filtered to remove DCC and a solvent mixture of isopropanol/hexane (1:20) was added to the filtrate to give product (0.27 g). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.76$ (1H, s), 7.57 (1H, d), 6.95 (1H, d, $J = 9.0$ Hz), 6.85 (1H, s), 3.95 (3H, t), 2.90 ppm (4H, s).

LO coumarin complex 19: A mixture of LO **15** (0.2 g, 0.05 mmol) and 7-methoxycoumarin-3-carboxylic succinimidyl ester (1.5 equiv, 0.08 g) was dissolved in a solution of 30% methanol/acetonitrile and triethylamine (1 equiv, 0.02 g) was added. The reaction mixture was stirred at room temperature for 2 h. The organic solvents were evaporated and the crude product was purified using preparative-HPLC to give **19** (0.24 g). ^{1}H NMR (500 MHz, $CDCl_3$, COSY, NOESY): $MetO_2^1$: $\delta = 4.76$ (1H, m, α), 2.51 (1H, m, β), 2.39 (1H, m, β), 3.40 (2H, m, γ), 3.87 (2H, s, SO_2CH_2), 2.87 (4H, m, $NHCH_2CH_2NH$), 7.79 (1H, brs, $NHCH_2CH_2NH$Coumarin), 8.90 (1H, m, $NHCH_2CH_2NH$Coumarin), 3.91 (3H, s, OCH_3), 8.79 (1H, s), 7.57 (1H, d, 8.6 Hz), 6.92 (1H, d, 8.6 Hz), 6.85 (1H, s), 7.31 ppm (1H, m, NH); Leu^2: $\delta = 3.83$ (1H, m, α), 1.93 (2H, m, β), 1.62 (1H, m, γ), 0.92 ppm (6H, d, 7 Hz, δMe); Ile^3: $\delta = 4.47$ (1H, m, α), 1.90 (1H, m, β), 1.49 (2H, m, γ), 1.07 (3H, d, 6.8 Hz, γMe), 0.82 ppm (3H, t, 6.8 Hz, δMe); Pro^4: $\delta = 3.78$ (1H, brs, α), 2.15 (1H, m, β), 1.64 (1H, m, β), 1.98 (1H, m, γ), 1.94 (1H, m, γ), 3.86 (1H, brs, δ), 3.61 ppm (1H, m, δ); Pro^5: $\delta = 4.11$ (1H, m, α), 1.93 (2H, m, β), 1.49 (1H, m, γ), 0.95 (1H, m, γ), 3.31 ppm (2H, m, δ); Phe^6: $\delta = 4.86$ (1H, m, α), 3.08 (1H, m, β), 2.98 (1H, m, β), 7.25 (3H, m, δ, ε), 7.14 ppm (2H, m, ζ); Phe^7:

$\delta = 3.92$ (1H, m, α), 3.31 (2H, m, β), 7.30 (2H, m, δ), 7.25 (1H, m, ε), 7.14 (2H, m, ζ) 7.71 ppm (1H, brs, NH); Val[8]: $\delta = 3.86$ (1H, m, α), 2.16 (1H, m, β), 0.90 ppm (6H, d, 7 Hz, γMe); Ile[9]: $\delta = 4.55$ (1H, m, α), 1.98 (1H, m, β), 1.51 (2H, m, γ), 0.95 (3H, d, 6.4 Hz, γMe), 0.84 (3H, t, 6.4 Hz, δMe), 6.78 ppm (1H, brs, NH); ^{13}C NMR (125 MHz, $CDCl_3$, HMQC, HMBC): $MetO_2$[1]: $\delta = 52.1$ (α), 25. 5 (β), 50.6 (γ), 58.4 (SO_2CH_2), 40.5 ($NHCH_2CH_2NH$Coumarin), 39.5 ($NHCH_2CH_2NH_2$), 56.2 (OCH_3), 165.3, 163.2, 161.8, 161.2, 157.0, 148.6, 131.3, 114.9, 114.2, 112.6, 100.6, 171.0 ppm (C=O); Leu[2]: $\delta = 61.8$ (α), 38.4 (β), 25.8 (γ), 23.1 (δMe), 21.9 (δMe), 171.7 ppm (C=O); Ile[3]: $\delta = 56.3$ (α), 36.6 (β), 25.1 (γ), 15.6 (γMe), 11.1 (δMe), 171.9 ppm (C=O); Pro[4]: $\delta = 56.2$ (α), 28.6 (β), 25.5 (γ), 48.1 (δ), 170.9 ppm (C=O); Pro[5]: $\delta = 61.1$ (α), 32.0 (β), 21.7 (γ), 47.3 (δ), 173.7 (C=O); Phe[6]: $\delta = 54.1$ (α), 35.5 (β), 136.3 (γ), 129.7 (δ), 129.0 (ε), 127.3 (ζ), 173.2 ppm (C=O); Phe[7]: $\delta = 57.3$ (α), 36.9 (β), 135.9 (γ), 129.1 (δ), 128.9 (ε), 127.3 (ζ), 175.4 ppm (C=O); Val[8]: $\delta = 56.2$ (α), 29.4 (β), 19.7 (γMe), 18.3 (γMe), 173.2 ppm (C=O); Ile[9]: $\delta = 59.1$ (α), 36.8 (β), 25.1 (γ), 16.1 (γMe), 11.3 (δMe), 173.0 ppm (C=O).

LO coumarin complex 20: Similarly **18** (0.2 g, 0.18 mmol) was coupled with 7-methoxycoumarin-3-carboxylic succinimidyl ester (1.5 equiv, 0.09 g) in the presence of triethylamine (1 equiv, 0.19 mg, 0.025 mL) in a solution of 30% methanol/acetonitrile to give **20** (0.25 g) by using the above procedure. ^{1}H NMR (500 MHz, $CDCl_3$, COSY, NOESY): $MetO_2$[1]: $\delta = 4.63$ (1H, m, α), 2.52 (1H, m, β), 2.17 (1H, m, β), 3.29 (1H, m, γ), 3.13 (1H, m, γ), 3.72 (2H, s, SO_2CH_2), 3.60 (2H, m, $NHCH_2CH_2NH$Coumarin), 3.49 (2H, m, $NHCH_2CH_2NH$Coumarin), 8.76 (1H, brs, $NHCH_2CH_2NH$Coumarin), 8.96 (1H, m, $NHCH_2CH_2NH$Coumarin), 3.87 (3H, s, OCH_3), 8.81 (1H, s), 7.63 (1H, d, 9 Hz), 6.86 (1H, d, 9 Hz), 6.80 (1H, s), 7.74 ppm (1H, m, NH); Leu[2]: $\delta = 4.33$ (1H, m, α), 2.33 (1H, m, β), 1.54 (1H, m, β), 1.84 (1H, m, γ), 0.83 ppm (6H, m, δMe); Val[3]: $\delta = 4.42$ (1H, m, α), 2.17 (1H, m, β), 1.02 (6H, m, γMe), 7.56 ppm (1H, brs, NH); Phe[4]: $\delta = 4.42$ (1H, m, α), 3.13 (1H, m, β), 3.06 (1H, m, β), 7.30–7.10 ppm (5H, m, δ, ε, ζ); Pro[5]: $\delta = 4.12$ (1H, m, α), 1.46 (2H, m, β), 1.59 (1H, m, γ), 1.46 (1H, m, γ), 3.29 ppm (2H, m, δ); Leu[6]: $\delta = 3.60$ (1H, m, α), 1.70 (2H, m, β), 1.39 (1H, m, γ), 0.93 (6H, m, δMe), 7.95 ppm (1H, d, 7.5, NH); Phe[7]: $\delta = 4.97$ (1H, m, α), 3.02 (2H, m, β), 7.30–7.10 ppm (5H, m, δ, ε, ζ); Ile[8]: $\delta = 3.90$ (1H, m, α), 1.76 (1H, m, β), 1.60 (2H, m, γ), 0.83 (3H, m, γMe), 0.93 (3H, m, δMe), 7.67 ppm (1H, brs, NH); ^{13}C NMR (125 MHz, $CDCl_3$, HMQC, HMBC): $MetO_2$[1]: $\delta = 52.0$ (α), 25.4 (β), 50.7 (γ), 48.2 (SO_2CH_2), 40.3 ($NHCH_2CH_2NH$Coumarin), 38.7 ($NHCH_2CH_2NH_2$), 56.1 (OCH_3), 165.1, 162.9, 162.9, 161.9, 156.8, 148.8, 131.7, 114.7, 114.0, 112.8, 100.4, 172.8 ppm (C=O); Leu[2]: $\delta = 63.5$ (α), 30.1 (β), 28.9 (γ), 22.9 (δMe), 21.7 (δMe), 172.8 (C=O); Val[3]: $\delta = 57.7$ (α), 30.9 (β), 19.5 (γMe), 18.4 (γMe), 171.9 ppm (C=O); Phe[4]: $\delta = 53.5$ (α), 36.0 (β), 138.1 (γ), 129.1 (δ), 128.9 (ε), 127.0 (ζ), 173.2 ppm (C=O); Pro[5]: $\delta = 60.2$ (α), 30.6 (β), 25.2 (γ), 46.4 (δ), 173.9 ppm (C=O); Leu[6]: $\delta = 61.2$ (α), 40.2 (β), 25.0 (γ), 22.7 (δMe), 21.6 (δMe), 171.3 ppm (C=O); Phe[7]: $\delta = 53.5$ (α), 37.6 (β), 137.7 (γ), 129.0 (δ), 128.4 (ε), 126.5 (ζ), 171.9 ppm (C=O); Ile[8]: $\delta = 58.7$ (α), 35.9 (β), 25.2 (γ), 16.1 (γMe), 11.5 (δMe), 171.3 ppm (C=O).

Tb complex of 19 (21): Compound **19** was co-dissolved with one equivalent of $TbCl_3$ in methanol and then refluxed overnight. Finally, the solvent was removed to obtain Tb complex (**21**). Putative Tb complex was confirmed by HPLC-MS. HPLC-MS analysis showed a peak at 767.8 $[M+Tb]^{2+}$ corresponding to a molecular formula, $C_{70}H_{95}N_{11}O_{16}STb$ of the Tb complex. Other compounds identified were $C_{70}H_{97}N_{11}O_{16}SCl_2Tb$ $[M+TbCl_2]^+$: 1608.5; $C_{70}H_{95}N_{11}O_{16}SNa$ $[M+Na]^+$: 1400.7

Eu complex of 19 (22): Compound **19** was co-dissolved with one equivalent of $EuCl_3$ in methanol and then refluxed overnight. Finally, solvent was removed to obtain Eu complex (**22**). Putative Eu complex was confirmed by an HPLC-MS peak at 764.8 $[M+Eu]^{2+}$ corresponding to a molecular formula, $C_{70}H_{94}N_{11}O_{16}SEu$ of the Eu complex and $C_{70}H_{97}N_{11}O_{16}SCl_2Eu$ $[M+EuCl_2]^+$: 1600.5.

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