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Protein–Inorganic Array Construction: Design and Synthesis of the Building Blocks

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Abstract: Herein we describe the design and synthesis of the first series of di-functional ligands for the directed construction of inorganic-protein frameworks. The synthesized ligands are composed of a metal-ion binding moiety (terpyridine-based) conjugated to an epoxysuccinyl peptide, known to covalently bind active cysteine proteases through the active-site cysteine. We explore and optimize two different conjugation chemistries between the di-functionalized metal-ion ligand and the epoxysuccinyl-containing peptide moiety: peptide-bond formation (with limited success) and Cu^{I}-catalysed click chemistry (with good results). Further, the complexation of the synthesized ligands with Fe^{II} and Ni^{II} ions is investigated: the di-functional ligands are confirmed to behave similarly to the parent terpyridine. As designed, the peptidic moiety does not interfere with the complexation reaction, in spite of the presence of two triazole rings that result from the click reaction. ES-MS together with NMR and UV/Vis studies establish the structure, the stoichiometry of the complexation reactions, as well as the conditions under which chemically sensitive peptide-containing polypyridine ligands can undergo the self-assembly process. These results establish the versatility of our approach and open the way to the synthesis of di-functional ligands containing more elaborated polypyridine ligands as well as affinity labels for different enzyme families. As such, this paper is the first step towards the construction of robust supramolecular species that cover a size-regime and organization level previously unexplored.

Keywords: click chemistry · inorganic–protein frameworks · peptides · self-assembly · terpyridines

Introduction

Metal-ion-directed self-assembly of polydentate ligands has generated a variety of metallosupramolecular entities bearing original physical and chemical properties, with discrete sizes and well-defined geometric shapes.[1] To further broaden the scope of the construction of discrete supramolecular species through metal-ion self-assembly-based methods we envisioned to combine the intrinsic efficiency of the self-assembly process with the efficiency and specificity of the conjugation of active enzymes to affinity labels. Such a process would result in supramolecular species, bearing both inorganic and proteic frameworks,[2] reaching sizes and complexity levels beyond the size-regime currently reached by metal-ion self-assembled molecules (usually bellow 5 nm).[3] To do so, we based our approach on ligands containing metal-ion binding units known for their complexation properties,[1c,4] conjugated with affinity labels that are known to bind specifically and covalently a specific amino acid in the active site of an active enzyme.[5]

We describe herein, as a first example in this series, the synthesis of ligands containing terpyridine as metal-ion complexing unit and epoxysuccinyl-based peptides as affinity labels well validated for cysteine proteases (Figure 1). The general principle along which we intend to use these ligands in order to build hybrid, protein–inorganic supramolecular structures is depicted in Figure 1.

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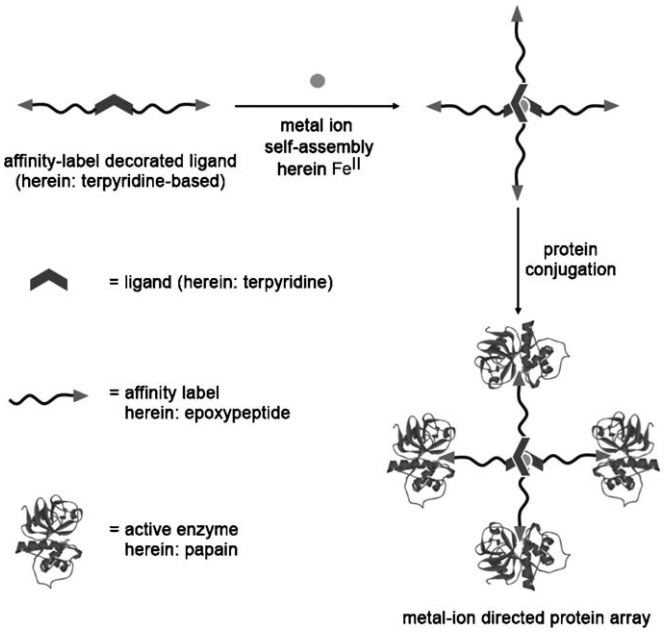
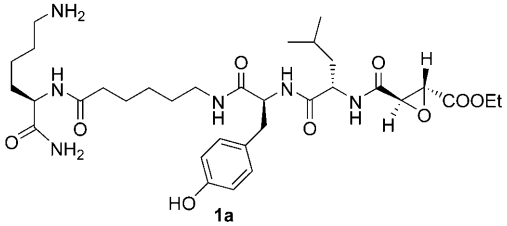


Figure 1. General principle for the construction of protein–inorganic hybrid compounds. An affinity-label compound is conjugated to a ligand known for its self-assembly properties. Complexation to metal-ions capable of expressing the molecular information encoded into the structure of the ligand yields a supramolecular complex decorated with affinity-labeling units. Their reaction with enzyme produces well organized protein–inorganic hybrid compounds.

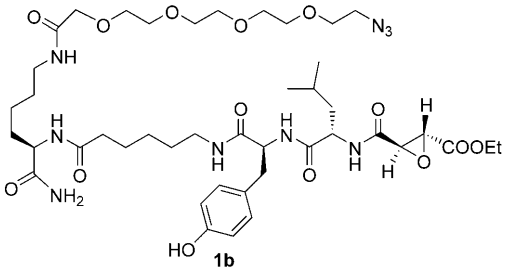
Abstract in German: *Im Folgenden beschreiben wir das gezielte Design und die Synthese di-funktionaler Liganden zum erstmaligen Aufbau supramolekularer Metall-Protein-Hybridarchitekturen. Die synthetisierten Liganden enthalten eine Metallionen-Bindungsstelle (auf Terpyridin-Basis), die mit einem Epoxysuccinyl-Peptid konjugiert wurde. Diese Peptide binden bekannterweise an die aktiven Cysteine im katalytischen Zentrum von Cystein-Proteasen. Wir untersuchen und optimieren zwei verschiedene Arten chemischer Konjugations-Systeme zwischen den di-funktionalen Metallionen-Liganden und dem Epoxysuccinyl-enthaltenden Peptidrest: Bildung einer Peptid-Bindung (mit geringem Erfolg) und Cu^{I}-katalysierte click Chemie (mit signifikantem Erfolg). Wie beabsichtigt, erfolgt die Komplexierung von Fe^{II}- und Ni^{II}-Ionen an den synthetisierten Liganden hochselektiv am Terpyridylrest und nicht an den Triazol-Ringen des Peptidrests, die aus der click Reaktion resultieren. Struktur, Stöchiometrie der Komplexbildung und Bedingungen für den Selbstorganisationsprozess der empfindlichen poly-Pyridyl-Peptid-Liganden wurden durch ESI-MS-, NMR- und UV-VIS-Untersuchungen dokumentiert. Diese Ergebnisse demonstrieren die Vielseitigkeit unseres neuartigen Ansatzes zur Synthese maßgeschneiderter di-funktionaler Liganden mit poly-Pyridyl-Resten und Affinity Label für unterschiedliche Enzymfamilien. Die Arbeit repräsentiert somit den ersten Schritt in der Entwicklung einer stabilen, supramolekularen Architektur in bisher unerreichter Größenordnung und Organisationsgrad.*

Results and Discussion

The first series of ligands on which we focused our attention contain 2,2′-6′,2″-terpyridine (terpy) as one of the most encountered units in the construction of supramolecular systems. Beyond supramolecular chemistry, compounds derived from this versatile heterocycle system found applications in molecular biology,[6] photochemistry,[7] polymer science,[8] enantioselective synthesis,[9] and pharmaceutical research.[10] This makes terpyridine (possibly bearing various functional groups) a building unit of choice in the context of potentially bio-compatible functions of the final assemblies. For covalent binding of the ligands to proteins with the efficiency of an enzymatic reaction we used epoxysuccinyl-terminated peptides, known to bind and covalently link (activity dependent) cysteine proteases of the papain family.[11] We chose to use a cysteine-protease affinity label, since some enzymes of this family, such as papain and bromelain, can be obtained in large quantities and are relatively easy to handle. Therefore, as a strong papain binding unit we used the short peptide sequence (YL), terminated by the chemical "war head", epoxide. As a linker between the terpyridine units and the YL–epoxide part, we investigated two spacer variants with different properties: 1) a hydrophobic chain consisting of 6-aminohexanoic acid (in peptide **1a**); and 2) a hydrophilic



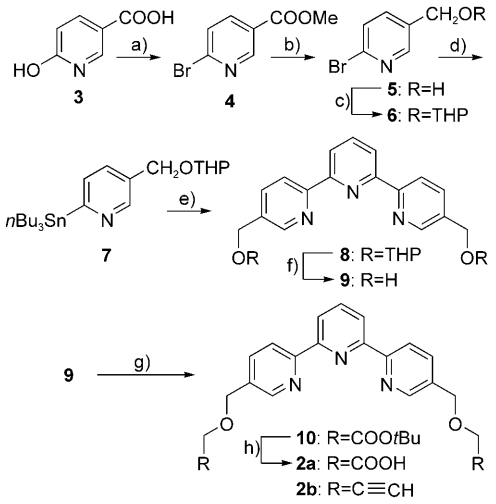
1a



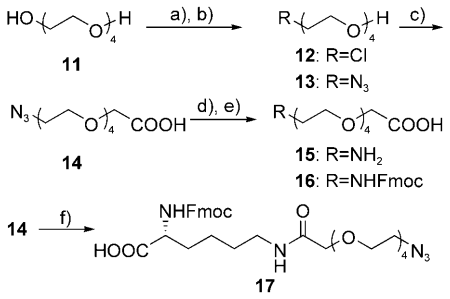
1b

chain consisting of a tetraethylene glycol-based linker for solubility and distance modulation reasons (in peptide **1b**). In order to find general conditions that allow a versatile synthetic methodology we investigated two different coupling chemistries for the conjugation between the metal-ion binding unit and the protein binding unit: amide bond forming reactions for the amino-terminated epoxysuccinyl peptide **1a** and click reactions for the azide-terminated epoxysuccinyl peptide **1b**.

Terpyridine units **2a** and **2b** were synthesized through the common intermediate diol **9** (Scheme 1). Optimized phase-



Scheme 1. Synthesis of the decorated terpyridine motifs: a) i: $POBr_3$ 170 °C, ii: MeOH, 90 %; b) $NaBH_4$, EtOH, 87 %; c) DHP, PTSA, $CHCl_3$, 81 %; d) *n*BuLi, THF, −78 °C, $(nBu)_3SnCl$, 75 %; e) 2,6-dibromopyridine, $[Pd(PPh_3)_4]$, toluene, 54 %; f) HCl, MeOH, 64 %; g) for **10**: *tert*-butyl bromoacetate, aq NaOH 50 %, TBAB, CH_2Cl_2, 48 %; for **2b** propargyl bromide, aq NaOH 50 %, TBAB, CH_2Cl_2, 85 %; h) 25 % TFA in CH_2Cl_2, 85 %.

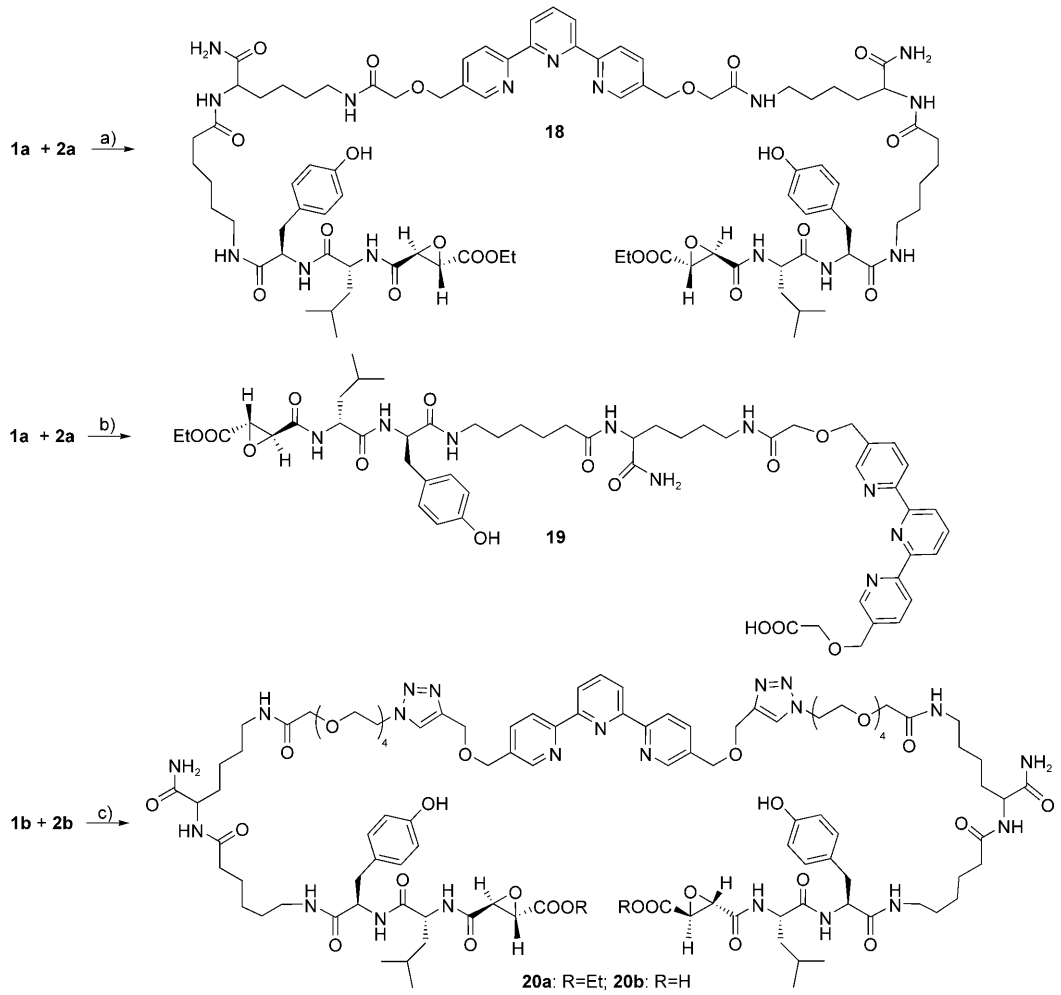


Scheme 2. Five-step synthesis of the polyethylene glycol spacer and azide-decorated lysine: a) $SOCl_2$, Py, $CHCl_3$, 31 %; b) NaN_3, NH_4Cl, H_2O, 73 %; c) bromoacetic acid, aq NaOH 50 %, TBAB, CH_2Cl_2, 76 %; d) H_2, Pd/C, MeOH, 82 %; e) Fmoc-Cl, $NaHCO_3$, dioxane, H_2O, 63 %; f) Fmoc-Lys(NH_2)-OH, HBTU, HOBt, DIPEA, 55 %.

transfer reactions[12] with either *tert*-butyl bromoacetate or propargyl bromide yielded (after TFA deprotection of the *tert*-butyl group) the carboxy-functionalized terpyridine **2a** and the alkyne functionalized terpyridine **2b**, respectively. We found that the diol **9** was best synthesized through the Pd-catalyzed coupling reaction[13] between the stannane **7** and 2,6-dibromopyridine followed by acid deprotection. This procedure provided diol **9** in 64 % isolated yield. In turn, the stannane **7** was synthesized in four steps starting with commercial hydroxynicotinic acid. Bromopyridine **4** was obtained by melting at 170 °C a mixture of neat hydroxynicotinic acid **3** with neat $POBr_3$ and subsequent MeOH quenching. $NaBH_4$ reduction of **4** followed by THP protection of the resulting alcohol **5** and the classical *n*-butyllithium/tri(*n*-butyl)tinchloride treatment yielded **7**.

The affinity labels, **1a** and **1b**, used for the two coupling chemistries investigated were synthesized by typical Fmoc-peptide solid-phase synthesis using the appropriate spacer units. Along with the commercially available amino acids (lysine, tyrosine, leucine and 6-aminohexanoic acid), the synthesis of **1a** and **1b** required the preparation of the epoxide unit and the azide-containing tetraethylene glycol spacer **17**. Ethyl (2*R*,3*R*)-*trans*-2,3-epoxysuccinate was synthesized starting from D-tartaric acid in a five-step synthesis as previously described.[14] Key compound **14** and the corresponding Fmoc-protected amino acid **16** were synthesized starting from tetraethylene glycol **11** (Scheme 2). Briefly, tetraethylene glycol **11** was desymmetrized by treatment with thionyl chloride and pyridine in chloroform[15] to obtain the monochloro tetraethylene glycol **12**. Substitution with sodium azide and further reaction with bromoacetic acid under phase-transfer conditions formed **14**. Azide reduction yielded tetraethylene glycol amino acid **15** and, through standard Fmoc protection conditions, compound **16**. For the completion of the synthesis of **17**, azide tetraethylene glycol **14** was reacted with the side chain amine of Fmoc-protected lysine to yield **17** in reasonable (55 %) yield.

With ligands **2a** and **2b** and peptides **1a** and **1b** in hand, we proceeded to optimize the conditions of the coupling reactions between the carboxy-terminated terpyridine and amino-terminated epoxysuccinyl peptide (Scheme 3). Amide coupling of the amino-terminated affinity label **1a** and the carboxy-terminated ligand **2a** was attempted by using different activation conditions for the carboxylic groups. As standard coupling procedure for peptide synthesis we first used stoichiometric amounts of activating reagents *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) and *N*-hydroxybenzotriazole (HOBt)) and three equivalents of the tertiary base (*N*,*N*-diisopropylethylamine (DIPEA)) in anhydrous DMF under inert conditions. This produced small amounts of the expected compound that could be observed in a complex mixture of products as judged from HPLC/MS data. Repeated attempts to synthesize the di-epoxide conjugates failed, despite using various coupling reagents known to be highly efficient for amide couplings (benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP), 2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU)) as well as slightly modified peptides. Noticeably, in the procedure using *N*,*N*′-diisopropylcarbodiimide (DIC)/HOBt as activating agents only the mono-epoxide conjugate compound **19** could be isolated in a modest yield (29 %), together with unreacted starting materials. On the other hand, exploration of the click reaction coupling chemistry, proved to be very efficient for our particular purpose (for a detailed study of the influence of polypyridine ligands on the click reaction see work of Finn[16]). We employed click reaction conditions using $[Cu(CH_3CN)_4]PF_6$ [tetrakis(acetonitrile)-copper(I) hexafluorophosphate] as catalyst, in degassed acetonitrile under inert atmosphere. Due to strong affinity of the terpyridine moiety towards copper ions, compound **2b** was first stirred for 30 min with one equivalent of the Cu^I



Scheme 3. Amide coupling between the affinity label **1a** and the carboxy-terminated terpyridine **2a** yields: a) under HBTU, HOBt, DIPEA conditions: low yield of di-epoxysuccinyl conjugated ligand **18** (traces), observed in HPLC-MS spectrum; or b) in DIC/HOBt conditions: mono-epoxysuccinyl conjugated ligand **19** in a modest yield (29%). Click reaction between the affinity label **1b** and the terpyridine alkyne **2b** yields (after decomplexation of the $[Cu^{II}(\mathbf{20a})]$ the di-epoxysuccinyl-conjugated ligand **20b** (see text) isolated from reaction in a very good yield; c) i: $[Cu(CH_3CN)_4]PF_6$, in dichloromethane/acetonitrile, 10 h, 60°C ($[Cu^{II}(\mathbf{20a})]$:79%, $[Cu^{II}(\mathbf{20a})_2]$: 6%), ii: aq HEEDTA 5% w/w (**20b**, 92%).

salt.[17] Following addition of the azide–epoxysuccinyl peptide, an extra 0.5 equivalents of copper(I) ions were added and the reaction was allowed to stir overnight at 60°C. The reaction between the epoxysuccinyl peptide **1b** and the dialkyne terpyridine **2b** yielded the desired compound **20a** as a mixture of Cu^{II} complexes (copper(II) diterpyridine $[Cu^{II}(\mathbf{20a})_2]$ and copper(II) monoterpyridine $[Cu^{II}(\mathbf{20a})]$) in a ratio of 1:12 as inferred from the HPLC/ES-MS spectrum of the crude of reaction (see Supporting Information). The complexes were separated by preparative HPLC and characterized as unique compounds. To obtain the free terpyridine–di-epoxide, the copper(II) complex $[Cu^{II}(\mathbf{20a})]$ was subjected to decomplexation by using a powerful chelating agent namely HEEDTA (2-(carboxymethyl-{2-[carboxymethyl-(2-hydroxyethyl)amino]ethyl}amino) acetic acid).[18] This yielded the metal-free elaborated affinity label **20b**, purified by HPLC. It should be noted that during the decomplexation step the ester groups were hydrolyzed to the free acid, a fact that is largely inconsequential to its reactivity towards papain.[19] The structural characteristics of **20b** were confirmed by NMR spectroscopy (see Supporting Information).

With compounds **19** and **20b** in hand, we set to test a crucial element of design in our overall strategy, namely to ensure that there is no interference relative to the metal-ion complexation between the metal-ion and protein-binding centers. Indeed, both amide-bond formation chemistry and click chemistry that we explored create, in close proximity to the terpyridine unit, functionalities that are potentially metal-ion complexing. Moreover, the importance of these control studies is underlined by recent reports[20] of the complexation of triazoles formed by click reactions with a variety of metal ions. With particular relevance to our work are the complexation studies performed by the Schibli group[21] on triazoles linked to biomolecules. In the light of these results, we set to demonstrate that the metal-ion complexing

properties of the model terpyridine triazole **21** are similar to those of terpyridine. Although, a priori, less susceptible of complexation interference, besides the complexing properties of **21**, we also studied the complexing properties of terpyridine diacid **2a** and terpyridine–di-alkyne **2b** (see Supporting Information). The simple triazole–terpyridine molecule (**21**, Figure 2) was synthesized by reaction of the triple-bond decorated terpyridine with a simple azide (N_3-CH_2-COO*t*Bu) in a manner analog to compounds **20a** and **20b**. The obtained product **21** was characterized by UV/Vis and NMR spectroscopy and ES-MS.

Terpyridine-based ligands are known to produce [M^{II}-(terpy)$_2$] complexes with octahedral metal ions. In particular, we studied the complexes of those ligands with Ni^{II} and Fe^{II} ions, because they complex terpyridine strongly and with different kinetic properties,[22] the Fe^{II} being particularly inert, whereas Ni^{II} complexes display a somewhat faster exchange between ligands (as a rule of thumb, a 5.4 mM solution of [Ni^{II}(terpy)$_2$] in acetonitrile requires 12 h at room temperature to reach the exchange equilibrium). The formed complexes were characterized by NMR and UV/Vis spectroscopy, and ES-MS. To establish the stoichiometry of the formed complexes, we performed UV/Vis spectroscopy titration experiments. In the case of the triazole-bearing terpyridine ligand **21** complexation with Fe^{II} we also performed ^{1}H NMR titration experiments in order to gather structural information about the structure of the complex. The general conclusion is that, as expected, all terpyridine-based ligands form [M^{II}(terpy)$_2$] complexes, thus validating our approach. When treated with increasing concentrations of Fe^{II}, ligand **21** displayed two absorption maxima at 325 nm (band characteristic for the conformational change in terpyridine from *trans/trans* conformation in ligand to the planar *cis/cis* conformation in the complex) and at 554 nm (band assigned to the metal–ligand charge transfer, MLCT) characteristic for [Fe^{II}(terpy)$_2$] complexes.[22] The increase in absorbance reached a plateau at a ratio of Fe^{II}/**21** = 1/2, indicating that the formed complex is composed of one Fe^{II} ion and two ligands **21**. ES-MS of the characteristic violet-colored complex displays a main peak at *m/z* = 711.5, corresponding to the molecular mass of [Fe^{II}(**21**)$_2$]$^{2+}$/2, a peak at *m/z* = 1509.2 that is due to the monocharged species [Fe^{II}(**21**)$_2BF_4$]$^+$, as well as lower mass fragments at *m/z* = 683.6, 655.5, 627.5 and 599.6 respectively, corresponding to the successive loss of

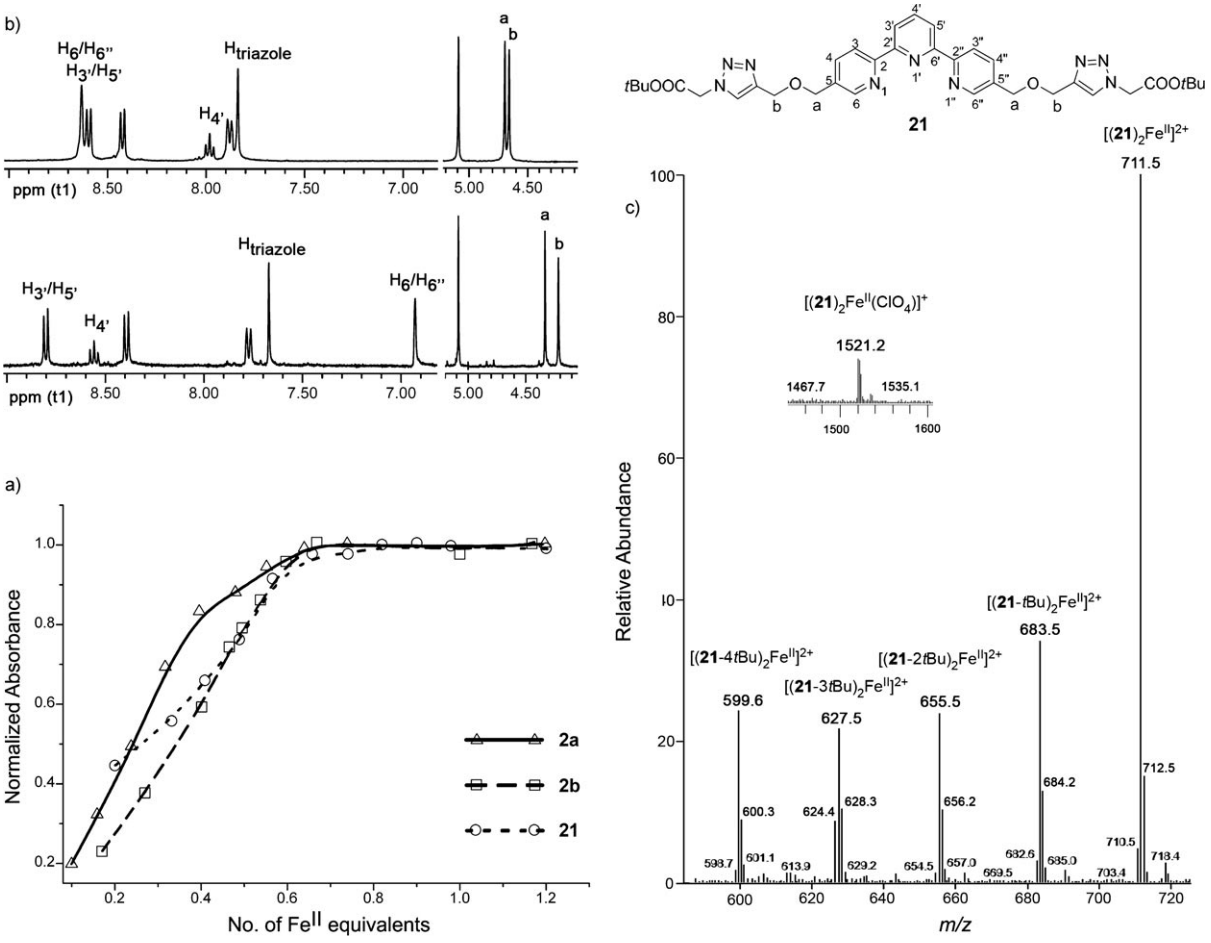


Figure 2. a) UV/Vis titration of **21**, **2a**, and **2b**, with Fe^{II} at 554 nm; b) ^{1}H NMR spectra of free ligand **21** (top) and of the [Fe^{II}(**21**)$_2$] complex (bottom); c) ES-MS of the [Fe^{II}(**21**)$_2$] complex. Further data (NMR, UV/Vis and ES-MS) corresponding to the complexation of **2a** and **2b** with Fe^{II} and Ni^{II} can be found in the Supporting Information.

the four *tert*-butyl groups from the ligands **21**. The same phenomenon could be observed in the case of the complex $[Fe^{II}(\mathbf{2b})_2]$, which loses propargyl fragments under the ionization conditions of the ES-MS. ^{1}H NMR studies of the $[Fe^{II}(\mathbf{21})_2]$ complex in [D$_3$]acetonitrile reveals several diagnostic characteristics. Noticeably, Fe^{II} complexation results in downfield shifts of protons $H_{3'}/H_{5'}$ ($\Delta\delta = 0.37$ ppm) and $H_{4'}$ ($\Delta\delta = 0.57$ ppm), which belong to the central pyridine ring, and a significant upfield shift ($\Delta\delta = 1.70$ ppm) of the protons $H_6/H_{6''}$ next to the nitrogen atoms $N_1/N_{1''}$ (Figure 2). This behavior,[23] especially the significant upfield shift of the $H_6/H_{6''}$ protons, is characteristic for the formation of a 2:1 complex and is due to the ring current of the second, perpendicularly placed terpyridine unit. This conclusion is further reinforced by the observation[23a] that the absence of the second terpyridine unit, in a 1:1 complex, results in a downfield shift of those protons. Moreover, following the Fe^{II} complexation of **21** we observe the upfield shift of the triazole proton ($\Delta\delta = 0.17$ ppm) as well as the upfield shift of the protons that belong to the two methylene groups (a and b, Figure 2) next to the terpyridine ($\Delta\delta = 0.19$ and 0.33 ppm). This indicates that these protons are in close proximity to the aromatic ring current (as would be the case in a classical $[Fe^{II}(terpy)_2]$ complex), suggesting a geometrical position of the triazole ring that excludes the nitrogen complexation to a Fe^{II} ion. Additionally, ^{1}H NMR titration of **21** with Fe^{II} reveals that the complexation reaction is complete at a molar ratio of $Fe^{II}/\mathbf{21} = 1/2$. Furthermore, during the titration experiments we could only observe signals belonging to the uncomplexed terpyridine and the $[Fe^{II}(\mathbf{21})_2]$ complex. Taken together, these data prove that the presence of the triazole in close proximity to the terpyridine does not affect the complexation of terpyridine with Fe^{II}. Recent data[24] show that somewhat similar compounds form $[Eu^{III}(terpy)_2]$ complexes, in which the nitrogen atoms of a tetrazole ring close to terpyridine are involved in the complexation of Eu^{III}, a fact that is not surprising in the light of the high coordination number of Eu^{III}.

Based on these results we performed the complexation of ligands **19** and **20b** with Fe^{II}. The reaction mixtures were analyzed by ES-MS and the complexes $[Fe^{II}(\mathbf{19})_2]$ as well as $[Fe^{II}(\mathbf{20b})_2]$ were purified by preparative HPLC. The ES-MS spectrum of Fe^{II} complex of **19** shows a main peak at $m/z =$ 1095.9 corresponding to the double charged species $[Fe^{II}(\mathbf{19})_2]^{2+}$. The ES-MS spectrum of Fe^{II} complex of ligand **20b** is more complex and exhibits peaks corresponding to triply charged species $[Fe^{II}(\mathbf{20b})_2H]^{3+}$, $[Fe^{II}(\mathbf{20b})_2Na]^{3+}$, $[Fe^{II}(\mathbf{20b})_2K]^{3+}$, $[Fe^{II}(\mathbf{20b})_2(Na)_2]^{3+}$, $[Fe^{II}(\mathbf{20b})_2NaK]^{3+}$, $[Fe^{II}(\mathbf{20b})_2(K)_2]^{3+}$, and $[Fe^{II}(\mathbf{20b})_2(H)_2(ClO_4)]^{3+}$ at m/z: 1475.5, 1482.6, 1488.1, 1488.9, 1495.4, 1499.9 and 1507.4, respectively, as well as quadruply charged species $[Fe^{II}(\mathbf{20b})_2(H)_2]^{4+}$, $[Fe^{II}(\mathbf{20b})_2HNa]^{4+}$, $[Fe^{II}(\mathbf{20b})_2HK]^{4+}$, $[Fe^{II}(\mathbf{20b})_2(Na)_2]^{4+}$, $[Fe^{II}(\mathbf{20b})_2NaK]^{4+}$, $[Fe^{II}(\mathbf{20b})_2(K)_2]^{4+}$, and $[Fe^{II}(\mathbf{20b})_2(H)_2K(ClO_4)]^{4+}$ at m/z: 1107.3, 1112.4, 1116.0, 1116.9, 1121.4, 1125.3, and 1140.7, respectively.

Conclusions

In conclusion we have developed an efficient method of synthesizing nitrogen-based ligands decorated with affinity labels. Although the initial attempts to couple the terpyridine moiety with the peptide-based affinity label by classical amide-bond formation chemistry yielded non-convincing results, a click reaction provided the desired ligand **20b** as well as, based on the new spacer **17**, the possibility to easily modulate the distance between the metal-ion complexing unit and the affinity labeling unit. This last fact is of particular importance considering the steric constraints that are related to our goal of attaching several enzyme molecules to one ligand. Setting the stage for the enzyme-reaction studies, we clearly established that the complexation of transition-metal ions, such as Fe^{II}, to ligand **21** does not interfere with the chemical functionalities (i.e., epoxysuccinyl peptides) introduced to covalently react in an activity-dependent manner with enzymes (in our case cysteine proteases of the papain family). Based on the synthetic methods developed herein we are currently expanding the repertoire of ligands coupled with affinity labels as well as studying the reaction of enzymes such as papain with the described ligands.

Experimental Section

General: All water-sensitive reactions were performed in anhydrous solvents and under a positive pressure of argon. Dry tetrahydrofuran (THF) and toluene were obtained by distillation under argon over sodium and benzophenone, while dimethylformamide (DMF) was distilled over diphosphorus pentoxide under argon. All other solvents, reagents and resins were purchased from commercial suppliers and used without further purification. Melting points were determined in open capillary tubes using an electric melting point STUART SMP3 apparatus and are uncorrected. The NMR spectra were recorded at room temperature on a JEOL-Delta 400, Bruker DPX-400 or Bruker-360 spectrometers. Chemical shifts (δ) are reported in parts per million (ppm) values using residual solvent peak as internal reference. Multiplicities are abbreviated as follows: br broad; s singlet; d doublet; t triplet; q quadruplet; and *m* multiplet. The mass spectra were obtained by electrospray ionization (ES) technique using a Finnigan LCQ instrument. The UV-Vis spectra were recorded on a Jasco V-630 spectrophotometer, using 10 mm quartz cell. Elemental analyses were performed on a CHN Elementar Vario EL apparatus. Thin-layer chromatography (TLC) was performed on aluminum oxide 60 F_{254} neutral plates or silica-gel-coated aluminium F_{254} plates from Merck. All plates were visualized by UV irradiation at 254 nm and/or staining with potassium permanganate. Preparative column chromatography was performed on Merck Aluminum oxide 60 [active basic (activity I) 70–230 mesh] or silica Merck 230. Reverse-phase HPLC analyses and purifications of the peptidic products were carried out on a VARIAN Pro Star 210 system, equipped with UV detection, using an analytical C18 VYDAC (300 Å, 4.6 mm × 150 mm, 5 μm) or a preparative C18 VYDAC (300 Å, 20 mm × 250 mm, 10 μm) columns, respectively and eluting with gradient mixtures of water (containing 0.1% TFA) and acetonitrile. The retention time (R_t) is given in minutes with the gradient in percentage of acetonitrile.

Methyl 6-bromo-nicotinate (4): Compound **3** (1 equiv, 10 g, 71.88 mmol) was mixed in a mortar with $POBr_3$ (2 equiv, 41.25 g, 143.77 mmol). The mixture was transferred to a round-bottomed flask and heated to melting (approx 170 °C, for 10 min) using a heat gun until it became a yellow-reddish oil. The resulted oil solidified at cooling and was carefully dissolved

in MeOH. The solvent was removed under reduced pressure and the residue was neutralized with $NaHCO_3$ and subsequently extracted with CH_2Cl_2. The organic phase was dried on $MgSO_4$ and evaporated to give **4** as light yellow solid (14 g, 90%). R_f=0.63 (silica, AcOEt/pentane 4:1); ^{1}H NMR (400 MHz, MeOD): δ=8.16 (d, ^4J=2.4 Hz, 1H; H_2), 7.99 (dd, ^3J=9.6 Hz, ^4J=2.4 Hz, 1H; H_4), 6.52 (d, ^3J=9.6 Hz, 1H; H_5), 3.85 ppm (s, 3H; CH_3).

2-Bromo-5-hydroxymethyl pyridine (5): Compound **4** (1 equiv, 5 g, 23 mmol) was dissolved in absolute ethanol (100 mL) and $NaBH_4$ (12 equiv, 10.5 g, 276 mmol) was slowly added. The reaction was stirred overnight at RT. The solvent was removed in vacuo and the reaction mixture was treated with KOH (1 M, 100 mL). Extraction with CH_2Cl_2 (8× 100 mL) afforded after drying over $MgSO_4$, evaporation and flash chromatography pure alcohol **5** (3.8 g, 87%). R_f=0.45 (silica, AcOEt/pentane 2:1); ^{1}H NMR (400 MHz, MeOD): δ=8.89 (d, ^4J=2.4 Hz, 1H; H_6), 8.21 (dd, ^3J=8.4 Hz, ^3J=2.4 Hz, 1H; H_4), 7.74 (d, ^3J=8.4 Hz, 1H; H_5), 3.94 ppm (s, 2H, CH_2).

2-Bromo-5-{[(tetrahydro-2*H*-pyran-2-yl)oxy]methyl}pyridine (6): Compound **5** (1 equiv, 2.5 g, 13.3 mmol) was suspended in $CHCl_3$ (15 mL) and 3,4-dihydro-2*H*-pyran (DHP) (1.3 equiv, 1.58 mL, 17.3 mmol) was added. A catalytic amount of *p*-TsOH (10 mol%) was also added to the reaction mixture. The reaction was stirred overnight at reflux. The solvent was removed in vacuo and the residue was subjected to column chromatography to afford the pure compound **6** as an yellow oil (2.9 g, 81%). R_f= 0.68 (silica, AcOEt/pentane 1:1); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ= 8.36 (d, ^4J=2.4 Hz, 1H; H_6), 7.73 (dd, ^3J=8.0 Hz, ^4J=2.4 Hz, 1H; H_4), 7.64 (d, ^3J=8.0 Hz, 1H; H_5), 4.68–4.65 (overlapped peaks, 2H; CH_2-O-C*H*-O, C*H*H-OTHP), 4.46 (d, ^2J=12.0 Hz, 1H; CH*H*-OTHP), 3.74 (ddd, ^2J=10.4 Hz, ^3J=5.2 Hz, $^3J'$=3.2 Hz, 1H; O-CH-O-C*H*H), 3.45 (ddd, ^2J= 10.4 Hz, ^3J=5.2 Hz, $^3J'$=5.2 Hz, 1H; O-CH-O-CH*H*), 1.70–1.63 (overlapped peaks, 2H; OTHP), 1.53–1.45 ppm (overlapped peaks, 6H; OTHP).

2-[Tri(*n*-butyl)stannyl]-5-{[(tetrahydro-2*H*-pyran-2-yl)oxy]methyl}pyridine (7): *n*BuLi (1.12 equiv, 2.55 mL, 4.4 mmol) was added in dry THF (10 mL) at −78°C, under Ar atmosphere. The protected alcohol **6** (1 equiv, 1 g, 3.67 mmol) was added dropwise to this solution. The mixture was allowed to stir for 45 min at −78°C and nBu_3SnCl (1.2 equiv, 1.2 mL, 4.41 mmol) was added. The reaction mixture was allowed to warm to RT overnight and the solvent was removed in vacuo. The resulted residue was partitioned between CH_2Cl_2 (20 mL) and water (10 mL). The aqueous phase was washed three times with CH_2Cl_2 (20 mL), then the combined organic phases were dried on $MgSO_4$ and evaporated. The product **7** (1.6 g, 75%) was used in the next step without any further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.74 (s, 1H; H_6), 7.51 (d, ^3J= 7.6 Hz, 1H; H_4), 7.39 (d, ^3J=7.6 Hz, 1H; H_5), 4.76 (d, ^2J=12.0 Hz, 1H; C*H*H-OTHP), 4.71 (t, ^3J=3.6 Hz, 1H; CH_2-O-C*H*-O), 4.47 (d, ^2J= 12.0 Hz, 1H; CH*H*-OTHP), 3.90 (ddd, ^2J=10.8 Hz, ^3J=5.6 Hz, $^3J'$= 3.2 Hz, 1H; O-CH-O-C*H*H), 3.45 (ddd, ^2J=10.8 Hz, ^3J=5.6 Hz, $^3J'$= 4.8 Hz, 1H; O-CH-O-CH*H*), 1.8–1.46 (overlapped peaks, 18H; OTHP, *n*Bu), 1.32 (q, ^3J=7.2 Hz, 6H; Bn), 0.9 ppm (t, ^3J=7.2 Hz, 9H; *n*Bu).

5,5″-Bis{[(tetrahydro-2*H*-pyran-2-yl)oxy]methyl}-2,2′-6′,2″-terpyridine (8): Dry toluene (50 mL) was added to a mixture of 2,6-dibromopyridine (1 equiv, 1.5 g, 6.3 mmol), stannane **7** (3 equiv, 9.5 g, 18.9 mmol) and tetrakis(triphenylphosphine)palladium(0) (1.8 g, 1.5 mmol, 24 mol%) under Ar atmosphere, and the mixture was refluxed overnight. The reaction was evaporated and treated with a mixture of AcOEt (50 mL) and concentrated aqueous KF (15 mL) under vigurous stirring for an hour. The white precipitate that formed was filtered and the biphasic system was separated. The organic layer was evaporated and subjected to chromatography (alox, $CHCl_3$/pentane 7:3) to afford 1.56 g (54%) of pure compound **8**. ^{1}H NMR (200 MHz, $CDCl_3$): δ=8.68 (d, ^4J=1.6 Hz, 2H; H_6, $H_{6''}$), 8.60 (d, ^3J=8.2 Hz, 2H; H_3, $H_{3''}$), 8.43 (d, ^3J=8.0 Hz, 2H; $H_{3'}$, $H_{5'}$), 7.94 (t, ^3J=7.8 Hz, 1H; $H_{4'}$), 7.86 (d, ^3J=8.2 Hz, ^4J=1.6 Hz, 2H; H_4, $H_{4''}$), 4.88 (d, ^2J=13.5 Hz, 2H; C*H*H-OTHP), 4.75 (t, ^3J=3.3 Hz, 2H; CH_2-O-C*H*-O), 4.61 (d, ^2J=13.5 Hz, 2H; CH*H*-OTHP), 4.01–3.91 (m, 2H; OTHP), 3.68–3.60 (m, 2H; OTHP), 1.76–1.68 ppm (m, 12H; OTHP); ^{13}C NMR (50 MHz, $CDCl_3$): δ=155.3, 155.0, 148.0, 137.6, 136.1, 133.7, 120.7, 120.6, 97.7, 66.0, 62.0, 30.3, 25.2, 19.0 ppm; elemental analysis calcd (%) for $C_{27}H_{31}N_3O_4$: C 70.26, H 6.77, N 9.10; found: C 69.95, H 6.79, N 8.98.

5,5″-Bis(hydroxymethyl)-2,2′-6′,2″terpyridine (9): Compound **8** (1 equiv, 100 mg, 0.34 mmol) was suspended in MeOH (20 mL) and 2 drops of 37% HCl were added. Reflux overnight followed by evaporation of the solvent (to 5 mL) and addition of 10% $NaHCO_3$ (5 mL) gave **9** as a white precipitate that was filtered and washed with water (120 mg, 64%). ^{1}H NMR (400 MHz, MeOD): δ=8.64 (s, 2H; H_6, $H_{6''}$), 8.59 (d, ^3J= 8.0 Hz, 2H; H_3, $H_{3''}$), 8.35 (d, ^3J=8.0 Hz, 2H; $H_{3'}$, $H_{5'}$), 8.02 (t, ^3J= 8.0 Hz, 1H; $H_{4'}$), 7.96 (d, ^3J=8.0 Hz, 2H; H_4, $H_{4''}$), 4.74 ppm (s, 4H; CH_2OH).

5,5″-Bis{[(*tert*-butoxycarbonyl)methoxy]methyl}-2,2′-6′,2″-terpyridine (10): The diol **9** (1 equiv, 140 mg, 0.48 mmol) was suspended in dichloromethane (2 mL) and the resulting mixture was cooled to 10°C. Aqueous solution of NaOH 50% (24 equiv, 240 mg, 11.52 mmol) and tetra *n*-butylammonium bromide (25 mol%) were added and the solution was allowed to stir for 30 min at 10°C. Bromo-*tert*-butylacetate (3 equiv, 294 mg, 1.44 mmol) was then added and the reaction was allowed to stir overnight at RT. The reaction mixture was diluted with water and CH_2Cl_2, and the two phases were separated. The organic phase was washed with brine, dried on $MgSO_4$, and evaporated. The residue was subjected to chromatography on aluminium oxide to yield the pure white compound **10** (120 mg, 48%). R_f=0.47 (alox, AcOEt/petroleum ether 1:3); m.p. 140–142°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.67 (d, ^4J= 2.4 Hz, 2H; H_6, $H_{6''}$), 8.61 (d, ^3J=8.8 Hz, 2H; H_3, $H_{3''}$), 8.44 (d, ^3J= 8.8 Hz, 2H; $H_{3'}$, $H_{5'}$), 7.94 (t, ^3J=8.8 Hz, 1H; $H_{4'}$), 7.92 (dd, ^3J=8.8 Hz, ^4J=2.4 Hz, 2H; H_4, $H_{4''}$), 4.72 (s, 4H; CH_2O), 4.05 (s, 4H; OCH_2COO*t*Bu), 1.49 ppm (s, 18H; *t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$): δ=169.3, 155.9, 155.1, 148.6, 137.9, 136.7, 132.9, 121.0, 120.9, 81.8, 70.6, 67.9, 28.1 ppm; MS (ES$^+$): *m/z*: 522.2 $[M+H]^+$, 466.2 $[M-t\text{Bu}+2H]^+$, 410.3 $[M-2t\text{Bu}+3H]^+$, 544.1 $[M+Na]^+$; elemental analysis calcd (%) for $C_{29}H_{35}N_3O_6$: C 66.78, H 6.76, N 8.06; found: C 66.94, H 6.52, N 7.81.

5,5″-Bis{[(carboxy)methoxy]methyl}-2,2′-6′,2″-terpyridine (2a): The diester **10** (120 mg, 0.23 mmol) and a mixture of 25% (v/v) trifluoroacetic acid in dichloromethane (10 mL) were stirred at RT overnight. The solvent was then removed in vacuo and the reaction mixture was triturated with ethyl ether. The precipitate was filtered and dried under reduced pressure to yield **2a** (80 mg, 85%). M.p. 190–192°C; ^{1}H NMR (400 MHz, MeOD): δ=8.74 (d, ^4J=2.0 Hz 2H; H_6, $H_{6''}$), 8.69 (d, ^3J=8.0 Hz, 2H; H_3, $H_{3''}$), 8.49 (d, ^3J=7.6 Hz, 2H; $H_{3'}$, $H_{5'}$), 8.11 (t, ^3J=7.6 Hz, 1H; $H_{4'}$), 8.05 (dd, ^3J=8.0 Hz, $^4J'$=2.0 Hz, 2H; H_4, $H_{4''}$), 4.77 (s, 4H; CH_2O), 4.25 (s, 4H; OCH_2COOH), 3.18 ppm (br, 1H; COO*H*); ^{13}C NMR (100 MHz, MeOD): δ=173.7, 152.9, 152.3, 146.4, 142.3, 141.1, 138.5, 124.5, 124.2, 70.7, 68.7 ppm; UV/Vis (MeCN) $\lambda_{max}(\varepsilon)$=285 (16000), 242 nm (15800 mol^{-1}m^{3}cm^{-1}); MS (ES$^+$): *m/z*: 410.3 $[M+H]^+$; elemental analysis calcd (%) for $C_{21}H_{19}N_3O_6$: C 61.61, H 4.78, N 10.26; found: C 61.33, H 4.47, N 9.96.

5,5″-Bis[(propynyloxy)methyl]-2,2′-6′,2″-terpyridine (2b): The diol **9** (1 equiv, 140 mg, 0.48 mmol) was suspended in dichloromethane (2 mL) and the resulted mixture was cooled to 10°C. Aqueous solution of NaOH 50% (24 equiv, 240 mg, 11.52 mmol,) and tetra-*n*-butylammonium bromide (25 mol%) were added and the solution was allowed to stir for 30 min at 10°C. Propargyl bromide (3 equiv, 168.5 mg, 1.44 mmol) was then added, and the reaction was allowed to stir overnight at RT. The reaction mixture was diluted with water and CH_2Cl_2, and the two phases were separated. The organic phase was washed with brine, dried on $MgSO_4$, and evapotated. The residue was subjected to chromatography on aluminium oxide to yield the pure white compound **2b** (150 mg, 85%). M.p. 105–106°C; R_f=0.50 (alox, AcOEt/petroleum ether 1:3); ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.66 (s, 2H; H_6, $H_{6''}$), 8.60 (d, ^3J= 8.8 Hz, 2H; H_3, $H_{3''}$), 8.44 (d, ^3J=8.8 Hz, 2H; $H_{3'}$, $H_{5'}$), 7.95 (t, ^3J= 8.8 Hz, 1H; $H_{4'}$), 7.85 (d, ^3J=8.8 Hz, 2H; H_4, $H_{4''}$), 4.71 (s, 4H; CH_2O), 4.24 (d, ^4J=2.4 Hz, 4H; OCH_2), 2.51 ppm (t, ^4J=2.4 Hz, 2H; C*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ=155.9, 155.1, 148.8, 137.9, 136.6, 132.9, 121.1, 120.9, 79.2, 75.1, 68.8, 57.4 ppm; UV/Vis (MeCN) $\lambda_{max}(\varepsilon)$=285 (18500), 242 nm (18000 mol^{-1}m^{3}cm^{-1}); MS (ES$^+$): *m/z*: 370.3 $[M+H]^+$, 185.8 $[M+2H]^{2+}$, 392.2 $[M+Na]^+$; elemental analysis calcd (%) for $C_{23}H_{19}N_3O_2$: C 74.78, H 5.18, N 11.37; found: C 74.97, H 5.41, N 11.04.

5,5″-Bis[({[(1-*tert*-butoxycarbonyl)methyl]-1*H*-1,2,3-triazol-4-yl}methoxy)methyl]-2,2′-6′,2″-terpyridine (21): $[Cu(CH_3CN)_4]PF_6$ (1 equiv, 40 mg, 0.108 mmol) was dissolved in degassed acetonitrile (1 mL) and added, under argon, to a solution of compound **1b** (39.8 mg, 0.108 mmol) in degassed acetonitrile (1 mL). The reaction mixture was allowed to stir for 30 min at RT. Upon addition of the copper(I) salt, the solution turned immediately to dark red, color characteristic for copper(I) complexes of terpyridine ligands. Azide (*tert*-butyl-2-azidoacetate, 2 equiv, 61.8 mg, 0.432 mmol) and copper(I) catalyst $[Cu(CH_3CN)_4]PF_6$, (0.5 equiv, 20.1 mg, 0.56 mmol) were added to this solution containing the copper(I) complex of **2b**, generated in situ. The reaction mixture was heated overnight at 60 °C. After cooling at RT, aqueous solution of hydroxy-2-ethylenediaminetriacetic acid (HEEDTA) 50 % w/w (1 mL) was added and the mixture was stirred at RT for 1 h. The two phases were separated, and the organic phase evaporated in vacuo. The residue was partitioned between water (10 mL) and CH_2Cl_2 (20 mL). The organic phase was washed with water (10 mL) and brine (10 mL), dried over $MgSO_4$, and evaporated. The crude product was further purified by flash chromatography to yield **21** as a white powder (53 mg, 72 %). $R_f = 0.23$ (alox, AcOEt/petroleum ether 1:1); m.p. 126–128 °C; ^{1}H NMR (400 MHz, CD_3CN): $\delta = 8.63$ (s, 2H; H_6, $H_{6''}$), 8.60 (d, $^3J = 8.4$ Hz, 2H; H_3, $H_{3''}$), 8.43 (d, $^3J = 7.6$ Hz, 2H; $H_{3'}$, $H_{5'}$), 7.99 (t, $^3J = 7.6$ Hz, 1H; $H_{4'}$), 7.88 (d, $^3J = 8.4$ Hz, 2H; H_4, $H_{4''}$), 7.84 (s, 2H; $CH_{triazole}$), 5.09 (s, 4H; N-CH_2), 4.70 (s, 4H; CH_2O), 4.66 (s, 4H; OCH_2), 1.46 ppm (s, 18H; *t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 167.0$, 156.1, 149.6, 145.4, 139.1, 137.5, 135.3, 125.6, 121.6, 121.4, 118.2, 83.7, 70.0, 64.3, 52.2, 28.1 ppm; UV/Vis (MeCN) $\lambda_{max}(\varepsilon) = 285$ (24 500), 242 nm (23 600 $mol^{-1}m^3cm^{-1}$); MS (ES$^+$): *m/z*: 684.4 $[M+H]^+$, 706.3 $[M+Na]^+$, 628.4 $[M-t\text{Bu}+H]^+$, 572.5 $[M-2t\text{Bu}+H]^+$; elemental analysis calcd (%) for $C_{35}H_{41}N_9O_6$: C 61.48, H 6.04, N 18.44; found: C 61.76, H 6.42, N 18.45.

11-Chloro-3,6,9-trioxa-1-undecanol (12): Tetraethylene glycol **11** (1 equiv, 50 g, 0.25 mol) was dissolved in chloroform (50 mL). Pyridine (1 equiv, 19.75 g, 0.25 mol) and thionyl chloride (1 equiv, 19 mL, 0.25 mol) were added to this solution, and the mixture was refluxed overnight. The solvent was removed in vacuo and the residue was partitioned between water (30 mL) and dichloromethane (100 mL). The two phases were separated; the organic phase was dried over $MgSO_4$ and evaporated. The resulted oil was distilled under reduced pressure (100 mbar). The fraction between 110–115 °C was then subjected to chromatography on silica gel (AcOEt) to yield **12** as colorless oil (16.4 g, 31 %). $R_f = 0.35$ (silica, AcOEt/petroleum ether 1:2); ^{1}H NMR (360 MHz, $CDCl_3$): $\delta = 3.84$–3.67 (overlapped peaks, 4H; H_{10}, H_{11}), 3.66–3.64 (overlapped peaks, 8H; H_4, H_5, H_7, H_8), 3.61–3.57 ppm (overlapped peaks, 4H; H_1, H_2); ^{13}C NMR (90 MHz, $CDCl_3$): $\delta = 72.4$, 71.3, 70.6, 70.5, 70.4, 70.2, 61.6, 42.6 ppm; MS (ES$^+$): *m/z*: 213.1 $[M+H]^+$, 235.2 $[M+Na]^+$.

11-Azido-3,6,9-trioxa-1-undecanol (13): Compound **12** (1 equiv, 9.5 g, 45 mmol) was dissolved in water (10 mL). Sodium azide (10 equiv, 29.25 g, 450 mmol) and NH_4Cl (12 equiv, 28.9 g, 540 mmol) were added to this solution and the mixture was heated to 60–80 °C, until complete conversion of the chloro-alcohol **12**, as monitored by ^{13}C NMR spectroscopy. The reaction mixture was then diluted with water and the product was extracted with ethyl acetate (7 × 50 mL). The product **13** (colorless oil) was used in the next step without any further purification (73 %). ^{1}H NMR (360 MHz, $CDCl_3$): $\delta = 3.71$ (overlapped peaks, 3H; H_{10}, OH), 3.67–3.65 (overlapped peaks, 10H; H_2, H_4, H_5, H_7, H_8), 3.60 (dt, $^3J = 5.0$ Hz, $^3J = 1.8$ Hz, 2H; H_1), 3.38 ppm (t, $^3J = 5.0$ Hz, 2H; H_{11}); ^{13}C NMR (90 MHz, $CDCl_3$): $\delta = 72.5$, 70.7, 70.6, 70.5, 70.3, 70.0, 61.7, 50.6 ppm; MS (ES$^+$): *m/z*: 220.1 $[M+H]^+$, 242.2 $[M+Na]^+$.

14-Azido-3,6,9,12-tetraoxa-1-tetradecanoic acid (14): Compound **13** (1 equiv, 7 g, 32 mmol,) was dissolved in dichloromethane (10 mL) and the solution was cooled to 10 °C. Aqueous solution of NaOH 50 % (12 equiv, 12 mmol) and tetra-*n*-butylammonium bromide (25 mol %) were added and the solution was allowed to stir for 30 min at 10 °C. Bromoacetic acid (1.5 equiv, 6.6 g, 48 mmol) was then added, and the reaction was allowed to stir overnight at RT. The reaction mixture was diluted with water, and three washings with dichloromethane (20 mL) followed by two washings with ethyl acetate (20 mL) were performed. The aqueous solution was acidified with concentrated HCl and the product was extracted with ethyl acetate (5 × 50 mL). The combined organic phases were washed with brine, dried on $MgSO_4$, and evaporated. The residue was subjected to chromatography on silica gel (DCM/MeOH 9:1) to yield **14** as colorless oil (76 %). $R_f = 0.42$ (silica, CH_2Cl_2/MeOH 9:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 5.38$ (br s, 1H; COOH), 3.94 (s, 2H; H_2), 3.70–3.60 (overlapped peaks, 14H; H_4, H_5, H_7, H_8, H_{10}, H_{11}, H_{13}), 3.38 ppm (t, $^3J = 5.2$ Hz, 2H; H_{14}); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 172.5, 70.5, 70.3, 70.2, 70.12, 70.08, 70.0, 69.6, 68.4, 50.3 ppm; MS (ES$^+$): *m/z*: 276.8 $[M+H]^+$.

14-Amino-3,6,9,12-tetraoxa-1-tetradecanoic acid (15): Compound **14** (6.5 g, 23.5 mmol) was dissolved in methanol (25 mL), flushed with nitrogen, and Pd/C (10 mol %, 10 % w/w) was added. Hydrogen filled balloons were used as hydrogen provider until completion of the reaction as monitored by TLC (CH_2Cl_2/MeOH 1:1). The reaction mixture was then filtered over Celite and the solvent removed in vacuo. The residue was subjected to column chromatography on silica gel (CH_2Cl_2/MeOH 1:1) to yield amino acid **15** as colorless oil (4.6 g, 82 %). $R_f = 0.15$ (silica, CH_2Cl_2/MeOH 1:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.92$ (s, 2H; CH_2COOH), 3.79 (t, $^3J = 4.4$ Hz, 2H; H_{13}), 3.67–3.60 (overlapped peaks, 12H; H_4, H_5, H_7, H_8, H_{10}, H_{11}), 3.13 ppm (t, $^3J = 4.4$ Hz, 2H; H_{14}); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 175.6$, 71.0, 70.1, 69.59, 69.57, 69.47, 69.35, 69.2, 67.3, 39.3 ppm; MS (ES$^+$): *m/z*: 252.3 $[M+H]^+$, 274.3 $[M+Na]^+$, 296.3 $[M-H+2Na]^+$.

14-*N*-{[(9′*H*-Fluoren-9′-yl)methoxy]carbonylamino}-3,6,9,12-tetraoxa-1-tetradecanoic acid (16): Compound **15** (1 equiv, 5 g, 21 mmol) was dissolved in a mixture of water/dioxane (1:1, v/v) and the pH was brought to about 8 using solid sodium bicarbonate (1.5 equiv, 2.65 g, 31.5 mmol). The mixture was then cooled to 0 °C and a solution of fluorenylmethyloxycarbonyl chloride (Fmoc-Cl, 1.2 equiv, 6.5 g, 25.2 mmol,) in 1,4-dioxane (10 mL) was added dropwise. The reaction was allowed to stir at RT overnight. The solvent was evaporated in vacuo and the remained aqueous phase was washed with ethyl acetate and acidified with HCl (1 N). Extraction with ethyl acetate (5 × 30 mL) was followed by drying of the combined organic phases over $MgSO_4$ and evaporation of the solvent. The residue was subjected to chromatography on silica gel (CH_2Cl_2/MeOH 9:1) to yield Fmoc-protected amino acid **16** as colorless oil (6.3 g, 63 %). The compound was azeotroped with toluene prior to use in SPPS. $R_f = 0.21$ (silica, CH_2Cl_2/MeOH 9:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.76$ (d, $^3J = 7.6$ Hz, 2H; H_{Ar}), 7.61 (d, $^3J = 7.6$ Hz, 2H; H_{Ar}), 7.39 (t, $^3J = 7.6$ Hz, 2H; H_{Ar}), 7.31 (t, $^3J = 7.6$ Hz, 2H; $H_{Ar.}$), 5.53 (br, 1H; NH), 4.40 (d, $^3J = 7.2$ Hz, 2H; CH_2(Fmoc)), 4.22 (t, $^3J = 7.2$ Hz, 1H; CH(Fmoc)), 4.13 (s, 2H; H_2), 3.71–3.59 (overlapped peaks, 14H; H_4, H_5, H_7, H_8, H_{10}, H_{11}, H_{13}), 3.39 ppm (q (overlapped td), $^3J = 9.6$ Hz, $^3J = 4.8$ Hz, 2H; H_{14}); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 175.8$, 159.0, 145.4, 142.7, 128.8, 128.2, 126.2, 120.9, 72.1, 71.9, 71.5, 71.4, 71.2, 71.1, 70.7, 67.6, 58.4, 41.7 ppm; MS (ES$^+$): *m/z*: 473.3 $[M+H]^+$, 496.4 $[M+Na]^+$.

***N*-α-{[(9′*H*-Fluoren-9′-yl)methoxy]carbonyl}-*N*-ε-[1″-azido-14″-oxo-3″,6″,9″,12″-tetraoxatetradecan]-L-lysine (17)**: Compound **14** (1 equiv, 400 mg, 1.3 mmol) was dissolved in anhydrous DMF, under argon, and HBTU (1 equiv, 544 mg, 1.3 mmol), HOBt (1 equiv, 194 mg, 1.3 mmol) and DIPEA (3 equiv, 0.70 mL, 3.9 mmol) were added and allowed to stir at RT for 10 min. Fmoc-Lys-COOH was then added (1.2 equiv, 590 mg, 1.56 mmol) and the reaction was allowed to stir overnight. The solvent was removed in vacuo and the product was purified by column chromatography (CH_2Cl_2/MeOH 9:1) to yield **17** as colorless oil (448 mg, 55 %). $R_f = 0.22$ (silica, CH_2Cl_2/MeOH 9:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta =$ 7.74 (d, $^3J = 7.2$ Hz, 2H; H_{Ar}), 7.58 (d, $^3J = 7.2$ Hz, 2H; H_{Ar}), 7.38 (t, $^3J = 7.2$ Hz, 2H; H_{Ar}), 7.29 (t, $^3J = 7.2$ Hz, 2H; H_{Ar}), 7.08 (br, 1H; NH), 5.77 (br, 1H; NH), 4.35 [d, $^3J = 6.4$ Hz, 2H; CH_2(Fmoc)], 4.19 [t, $^3J = 6.4$ Hz, 1H; CH(Fmoc)], 3.97 (s, 2H; H_{13}), 3.71–3.61 (overlapped peaks, 15H; H_2, H_4, H_5, H_7, H_8, H_{10}, H_{11}, H_{20}), 3.37 (t, $^3J = 5.2$ Hz, 2H; H_1), 3.16 (td, $^3J = 7.2$ Hz, $^3J' = 2$ Hz, 2H; H_{16}), 1.82–1.40 ppm (overlapped peaks, 6H; H_{17}, H_{18}, H_{19}); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 174.9$, 170.9, 161.9, 143.8, 141.2, 127.7, 127.1, 125.1, 119.9, 70.4, 70.3, 70.1, 69.85, 69.7, 69.5, 55.5, 50.5, 47.1, 43.5, 27.4, 31.5, 18.4, 17.0 ppm; MS (ES$^+$): *m/z*: 628.2 $[M+H]^+$.

Solid-phase synthesis of the amino-terminated peptide H_2N-Lys-Ahx-Tyr-Leu-epoxide 1a: Solid-phase peptide synthesis was performed on Rink amide MBHA (100 mg, loading 0.72 mmol g^{-1}). Unless otherwise stated the subsequent building blocks were introduced using Fmoc-amino acid/HBTU/HOBt/DIPEA (4:4:4:12 equiv) with respect to the loading reported by the supplier) in DMF (approximately 0.2 M) for 1–2 h at RT. Fmoc protecting groups were removed with piperidine/DMF 1:4 (2×10 min). Ethyl (2*R*,3*R*)-*trans*-2,3-epoxysuccinate (2 equiv) was coupled with DIC/HOBt (2:2 equiv) in DMF and the reaction shaken for 1 h. The peptide was cleaved from the resin using a cleavage cocktail (1 mL; 95% TFA, 2.5% water, 2.5% triisopropylsilane) and allowed to shake for 2 h. Ice-cold diethyl ether (15 mL) was used to precipitate the product. The resin was washed with TFA (1.5 mL) and the solution was collected separately in ice-cold diethyl ether. The solid was centrifuged, separated from diethyl ether and dissolved in a minimal amount of acetonitrile–water (0.1% TFA). The product was analyzed on a C18 reverse phase HPLC column using a linear gradient of 15–90% water (0.1% TFA)/acetonitrile and purified on a corresponding preparative C18 reverse-phase HPLC column, using the same gradient. Fractions containing the product were pooled, frozen and lyophilized to obtain **1a** as a white powder (31 mg, 63%). R_t = 8.62 min (RP-C18, 15–90% water (0.1% TFA)/acetonitrile); MS (ES^+): *m/z* calcd for for $C_{33}H_{52}N_6O_9$ $[M+H]^+$: 677.38; found: 677.4 $[M+H]^+$, 700.5 $[M+Na]^+$, 810.2 $[M+Na+TFA]^+$, 829.0 $[M+K+TFA]^+$.

Solid-phase synthesis of the azide-terminated peptide Lys(TegN$_3$)-Ahx-Tyr-Leu-epoxide 1b

Procedure A: Peptide **1b** was synthesized on of Rink amide MBHA (100 mg, loading 0.72 mmol $g)^{-1}$, as described for peptide **1a**. We used as first amino acid commercial Fmoc-Lys(Mtt)-OH and the corresponding amount of coupling reagents. Prior to the *N*-terminal Fmoc group deprotection, the Mtt group was deprotected using a solution 1% TFA in DCM (2 mL, 2 min×7) and compound **14** was coupled with HBTU, HOBt, DIPEA (2:2:2:4 equiv) for 2 h. The synthesis was further performed as described for peptide **1a**; (36 mg, 53%).

Procedure B: Peptide **1b** was synthesized on Rink amide MBHA (100 mg, loading 0.72 mmol $g)^{-1}$, as described for peptide **1a**. We used as first amino acid the Fmoc-Lys(TegN$_3$)-OH **17** and the corresponding amount of coupling reagents HBTU, HOBt, DIPEA (2:2.2:6 equiv). After complete attachment to the resin the synthesis was further performed as described for peptide **1a** (31 mg, 46%). White powder, R_t = 8.92 min (RP-C18, 25–60% water (0.1% TFA)/acetonitrile); MS (ES^+): *m/z* calcd for for $C_{43}H_{69}N_9O_{14}$ $[M+H]^+$: 936.06; found 936.4 $[M+H]^+$, 958.4 $[M+Na]^+$.

Solution-phase synthesis of compound 19 through amide coupling between the carboxy terminated ligand 2a and the amino-terminated affinity label 1a: Carboxy-terminated ligand **2a** (1 equiv, 2.53 mg, 6.2 μmol), DIC (1 equiv, 1.95 mg, 2.5 μL, 12.52 μmol) and HOBt (1 equiv, 1.69 mg, 12.52 μmol) were dissolved in anhydrous DMF (0.3 mL) under argon and allowed to stir at RT for 5 min. Dried amino-terminated epoxysuccinyl peptide **2a** (1.25 equiv, 10.64 mg, 15.67 μmol) was dissolved in a minimum amount of anhydrous DMF and added to the reaction mixture. The reaction mixture was allowed to stir until complete conversion of the diacid, as monitored by HPLC. The solvent was removed in vacuo and the crude of reaction was analyzed on a C18 reverse phase HPLC column using a linear gradient of water (0.1% TFA)/acetonitrile and purified on a corresponding preparative C18 reverse phase HPLC column (see General), using the same gradient. Fractions containing the mono-epoxide product were pooled, frozen and lyophilized to obtain 19 as white powder (1.9 mg, 29%). R_t = 15.2 min (RP-C18, 5–35% water (0.1% TFA)/acetonitrile); MS (ES^+): *m/z* calcd for $C_{54}H_{69}N_9O_{14}$ 1068.5 $[M+H]^+$; found: 1068.7 $[M+H]^+$, 1090.7 $[M+Na]^+$, 1106.5 $[M+K]^+$.

Solution-phase synthesis of compound 20 through click reaction between the ethynyl terminated ligand 1b and the azide-terminated affinity label 1b: $[Cu(CH_3CN)_4]PF_6$ (1 equiv, 1.1 mg, 2.8 μmol) dissolved in degassed acetonitrile (30 μL) was added under argon to a degassed solution of compound **2b** (1 equiv, 1.0 mg, 2.8 μmol) in acetonitrile/water (6:1, 0.5 mL), and the reaction mixture was allowed to stir for 30 min at RT. Upon addition of the copper(I) salt the solution turned immediately dark red, characteristic for copper(I) complexes of terpyridine ligands. Peptide **1b** (1.18 equiv, 6.2 mg, 6.64 μmol) and copper(I) catalyst $[Cu(CH_3CN)_4]PF_6$, (0.5 equiv, 0.5 mg, 1.4 μmol) were added to the solution containing the copper(I) complex of **2b**, generated in situ. The reaction mixture was allowed to stir at 60 °C until complete conversion of the copper(I) complex of **2b** (10 h), as monitored by HPLC. The solvent was removed in vacuo and the crude product was purified by preparative HPLC on a C18 reverse phase column using a linear gradient of water (0.1% TFA)/acetonitrile.

Complex $[Cu^{II}(20a)][PF_6]_2$: Green powder (5.3 mg, 79%); R_t = 10.09 min (RP-C18, 25–50% water (0.1% TFA)/acetonitrile); MS (ES^+): *m/z* calcd for $[Cu^{II}(\mathbf{20a})] = [C_{111}H_{159}CuN_{19}O_{30}]$ 2303.1; found *m/z*: 1152.0 $[(\mathbf{20a})+Cu^{II}]^{2+}$, 1162.9 $[(\mathbf{20a})+Cu^{II}+Na]^{2+}$, 1228.5 $[(\mathbf{20a})+Cu^{II}+H+(PF_6)]^{2+}$, 762.9 $[(\mathbf{20a})+Cu^{II}+2H]^{3+}$.

Complex $[Cu^{II}(20a)_2][PF_6]_2$: Green powder (0.8 mg, 6%); R_t = 7.56 min (RP-C18, 25–50% water (0.1% TFA)/acetonitrile); MS (ES^+): *m/z* calcd for $[Cu^{II}(\mathbf{20a})_2] = [C_{222}H_{318}CuO_{60}N_{38}]$ 4542.6; found *m/z*: 1137.9 $[(\mathbf{20a})_2+Cu^{II}+4H]^{4+}$, 1148.6 $[(\mathbf{20a})_2+Cu^{II}+2H+2Na]^{4+}$, 1156.8 $[(\mathbf{20a})_2+Cu^{II}+2K+H]^{4+}$, 766.7 $[(\mathbf{20a})_2+Cu\ Cu^{II}+Na+K+2H]^{6+}$.

Decomplexation of $[Cu^{II}(20a)][PF_6]_2$: HEEDTA (100 μL, of 5% solution w/w) was added to a solution of complex $[Cu^{II}(\mathbf{20})]$ in acetonitrile/water (0.72 mM, 900 μL, 2:8) and the reaction was monitored by HPLC. Complete decomplexation was achieved in 30 min. at RT. The reaction was quenched with a solution of 5% TFA in water (10 μL). Without any workup, the reaction mixture was immediately purified by preparative HPLC on a C18 reverse phase column using a linear gradient of water (0.1% TFA)/acetonitrile to yield the pure white compound **20** (92%). R_t = 10.6 min (RP-C18, 15–60% water (0.1% TFA)/acetonitrile); ^1H NMR (400 MHz, MeOD/D_2O = 8:2): δ = 8.72 (s, 2H; H_6, $H_{6''}$), 8.65 (d, ^3J = 7.6 Hz, 2H; H_3, $H_{3''}$), 8.44 (d, ^3J = 8.0 Hz, 2H; $H_{3'}$, $H_{5'}$), 8.15–8.00 (overlapped peaks, 5H; $H_{4'}$, H_4, $H_{4''}$, $CH_{triazole}$), 7.04 (d, ^3J = 8.4 Hz, 2H; H_{Ar-Tyr}), 7.02 (d, ^3J = 8.4 Hz, 2H; H_{Ar-Tyr}), 6.71 (d, ^3J = 8.4 Hz, 2H; H_{Ar-Tyr}), 6.69 (d, ^3J = 8.4 Hz, 2H; H_{Ar-}Tyr), 5.0–0.8 (overlapped peaks, 126H); MS (ES^+): *m/z* calcd for $C_{111}H_{159}N_{19}O_{30}$: 2183.5; found: 1093.4 $[M+2H]^{2+}$, 1104.3 $[M+Na+H]^{2+}$, 1112.4 $[M+K+H]^{2+}$, 1124.5 $[M+Na+K]^{2+}$, 1131.5 $[M+2K]^{2+}$, 1084.7 $[M-H_2O+2H]^{2+}$.

General procedure for complexation of the terpyridine-based ligands with metal salts: A solution of the appropriate metal salt in acetonitrile (0.5 equiv) was added to a solution of the terpyridine-based ligand (1 equiv) in acetonitrile (5 mL), and the mixture was stirred for 30 min. The solvent was removed in vacuo and the product was washed with water and crystallized with diethyl ether before use.

$[Fe^{II}(20b)_2](ClO_4)_2$: The complexation was performed as described in general procedure using a solution ligand **21** (0.36 mM, 300 μL) and a solution $Fe(ClO_4)_2$ (1.08 mM, 300 μL). The product was purified by preparative HPLC on a C18 reverse phase column using a linear gradient of water/acetonitrile. Violet powder (94%); MS (ES^+): *m/z* calcd for $[Fe^{II}(\mathbf{20b})_2] = [C_{214}H_{302}FeN_{38}O_{60}]$: 4422.7; found: 1475.5 $[(\mathbf{20b})_2+Fe^{II}+H]^{3+}$, 1482.6 $[(\mathbf{20b})_2+Fe^{II}+Na]^{3+}$, 1488.1 $[(\mathbf{20b})_2+Fe^{II}+K]^{3+}$, 1488.9 $[(\mathbf{20b})_2+Fe^{II}+2Na]^{3+}$, 1495.4 $[(\mathbf{20b})_2+Fe^{II}+Na+K]^{3+}$, 1499.9 $[(\mathbf{20b})_2+Fe^{II}+2K]^{3+}$, 1507.4 $[(\mathbf{20b})_2+Fe^{II}+2H+ClO_4]^{3+}$, 1107.3 $[(\mathbf{20b})_2+Fe^{II}+2H]^{4+}$, 1112.4 $[(\mathbf{20b})_2+Fe^{II}+H+Na]^{4+}$, 1116.0 $[(\mathbf{20b})_2+Fe^{II}+H+K]^{4+}$, 1116.9 $[(\mathbf{20b})_2+Fe^{II}+2Na]^{4+}$, 1121.4 $[(\mathbf{20b})_2+Fe^{II}+Na+K]^{4+}$, 1125.3 $[(\mathbf{20b})_2+Fe^{II}+2K_2]^{4+}$, 1140.7 $[(\mathbf{20b})_2+Fe^{II}+2H+K+ClO_4]^{4+}$.

$[Fe^{II}(2a)_2](ClO_4)_2$: Violet powder (6 mg, 92%); ^1H NMR (400 MHz, [D_3]MeCN): δ = 8.81 (d, ^3J = 8.8 Hz, 4H; $H_{3'}$, $H_{5'}$), 8.60 (t, ^3J = 8.8 Hz, 2H; $H_{4'}$), 8.38 (d, ^3J = 9.2 Hz, 4H; H_3, $H_{3''}$), 7.80 (d, ^3J = 9.2 Hz, 4H; H_4, $H_{4''}$), 6.94 (s, 4H; H_6, $H_{6''}$), 4.19 (s, 8H; CH_2O), 3.85 ppm (s, 8H; CH_2COOH); MS (ES^+): *m/z* calcd for $[Fe^{II}(\mathbf{2a})_2] = [C_{42}H_{38}FeN_6O_{12}]$: 874.63; found: 437.5 $[(\mathbf{2a})_2+Fe^{II}]^{2+}$, 415.6 $[(\mathbf{2a}\text{-}CO_2)_2+Fe^{II}]^{2+}$, 985.1 $[(\mathbf{2a}\text{-}2CO_2)_2+Fe^{II}+ClO_4]^{+}$, 1073.0 $[(\mathbf{2a})_2+Fe^{II}+2(ClO_4)+H]^{+}$.

$[Fe^{II}(2b)_2](ClO_4)_2$: Violet powder (6.1 mg, 94%); ^1H NMR (360 MHz, [D_3]MeCN): δ = 8.86 (d, ^3J = 8.0 Hz, 4H; $H_{3'}$, $H_{5'}$), 8.65 (t, ^3J = 8.0 Hz, 2H; $H_{4'}$), 8.41 (d, ^3J = 8.4 Hz, 4H; H_3, $H_{3''}$), 7.80 (d, ^3J = 8.4 Hz, 4H; H_4, $H_{4''}$), 6.89 (s, 4H; H_6, $H_{6''}$), 4.18 (s, 8H; CH_2O), 3.92 (d, ^4J = 2.4 Hz, 8H; CH_2), 2.59 ppm (t, ^4J = 2.4 Hz, 4H; CH); MS (ES^+): *m/z* calcd for $[Fe^{II}(\mathbf{1b})_2] = [C_{46}H_{38}FeN_6O_4]$: 794.2; found: 397.4 $[(\mathbf{2b})_2+Fe^{II}]^{2+}$, 880.9 $[(\mathbf{2b})_2+Fe^{II}+(BF_4)]^{+}$, 377.4 $[(\mathbf{2b}-CH_2CCH)_2+Fe^{II}]^{2+}$, 357.4 [{**2b**–2-

$(CH_2CCH)\}_2+Fe^{II}]^{2+}$, 337.4 [{**2b**−3$(CH_2CCH)\}_2+Fe^{II}]^{2+}$, 317.4 [{**2b**−4-$(CH_2CCH)\}_2+Fe^{II}]^{2+}$.

$[Ni^{II}(1b)_2](ClO_4)_2$: Green powder (6.7 mg, 87%); MS (ES^+): *m*/*z* calcd for $[Fe^{II}(\mathbf{2b})_2]=[C_{46}H_{38}Cl_2N_6NiO_{12}]$: 994.13; found: 398.7 $[(\mathbf{2b})_2+Ni^{II}]^{2+}$, 378.6 [{**2b**−(CH2CCH)$\}_2+Ni^{II}]^{2+}$, 358.6 [[**2b**−2$(CH_2CCH)\}_2+Ni^{II}]^{2+}$, 338.6 [{**2b**−3(CH2CCH)$\}_2+Ni^{II}]^{2+}$, 318.5 [{**2b**−4$(CH_2CCH)\}_2+Ni^{II}]^{2+}$, 895.1 $[(\mathbf{2b})_2+Ni^{II}+(ClO_4)]^+$.

$[Fe^{II}(21)_2](ClO_4)_2$: Violet powder (11 mg, 96%); ^1H NMR (400 MHz, $[D_3]$MeCN): $\delta=8.80$ (d, $^3J=8.0$ Hz, 4H; $H_{3'}$, $H_{5'}$), 8.56 (t, $^3J=8.0$ Hz, 2H; $H_{4'}$), 8.39 (d, $^3J=8.4$ Hz, 4H; H_3, $H_{3''}$), 7.77 (d, $^3J=8.4$ Hz, 4H; H_4, $H_{4''}$), 7.67 (s, 4H; $CH_{triazole}$), 6.93 (s, 4H; H_6, $H_{6''}$), 5.08 (s, 8H; N-CH_2), 4.33 (s, 8H; CH_2O); 4.21 (s, 8H; OCH_2), 1.47 ppm (s, 36H; *t*Bu); MS (ES^+): *m*/*z* calcd for $[Fe^{II}(21)_2]=[C_{70}H_{82}FeN_{18}O_{12}]$: 1423.3; found: 711.5 $[(\mathbf{21})_2+Fe^{II}]^{2+}$, 683.5 $[(\mathbf{21}-t\mathrm{Bu})_2+Fe^{II}]^{2+}$, 655.5 $[(\mathbf{21}-2t\mathrm{Bu})_2+Fe^{II}]^{2+}$, 627.5 $[(\mathbf{21}-3t\mathrm{Bu})_2+Fe^{II}]^{2+}$, 599.6 $[(\mathbf{21}-4t\mathrm{Bu})_2+Fe^{II}]^{2+}$, 1521.2 $[(\mathbf{21})_2+Fe^{II}+(ClO_4))]^+$.

$[Ni^{II}(21)_2](ClO_4)_2$: Green powder (10.1 mg, 89%); MS (ES^+): *m*/*z* calcd for $[Ni^{II}(21)_2]^{2+}=[C_{70}H_{82}NiN_{18}O_{12}]^{2+}$: 1426.2; found: 712.6 $[(\mathbf{21})_2+Ni^{II}]^{2+}$, 684.6 $[(\mathbf{21}-t\mathrm{Bu})_2+Ni^{II}]^{2+}$, 656.5 $[(\mathbf{21}-2t\mathrm{Bu})_2+Ni^{II}]^{2+}$, 628.5 $[(\mathbf{21}-3t\mathrm{Bu})_2+Ni^{II}]^{2+}$, 600.6 $[(\mathbf{21}-4t\mathrm{Bu})_2+Ni^{II}]^{2+}$, 1523.3 $[(\mathbf{21})_2+Ni^{II}+(ClO_4))]^+$.

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