Spectroscopic and Electrochemical Studies of Ferrocenyl Triazole Amino Acid and Peptide Bioconjugates Synthesized by Click Chemistry

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Ferrocenyl triazole amino acid (L-leucine methyl ester (**2**, **5**), L-phenylalanine methyl ester (**3**, **7**), L-alanine methyl ester (**4**), L-valine *tert*-butyl ester (**6**), L-proline methyl ester (**8**)) and peptide derivatives ([Leu^5]-enkephalin (**9**, **10**)) were prepared by Cu(I)-catalyzed [3+2] cycloaddition of azidoferrocene and 1,1′-diazidoferrocene with the corresponding alkyne-modified amino acids and peptide. A purity of >95% for the peptide conjugates was confirmed by HPLC. All new compounds were comprehensively characterized by elemental analysis, mass spectrometry (FAB and ESI-MS, including high-resolution MS), IR, and multinuclear 1D and 2D NMR spectroscopy. Solution structures were studied by circular dichroism (CD) and NMR spectroscopy, showing that compounds **5**, **6**, and **7** form intramolecular hydrogen bonds (IHBs) in noncoordinating solvents. Electrochemical studies show reversible processes of the redox couple Fc^0/Fc^+ (Fc = ferrocenyl) for compounds **2**−**9**, whereas compound **10** exhibits an irreversible oxidation. A good correlation between the diffusion coefficients as determined by electrochemical methods and the molecular weight was established.

Introduction

Bioconjugates of ferrocenecarboxylic acid, 1,1′-ferrocenedicarboxylic acid, 1,1′-diaminoferrocene, and the unnatural ferrocene-containing amino acid 1′-aminoferrocene-1-carboxylic acid (Fca) with amino acids and peptides have been and are being extensively investigated for possible biological applications and as structural mimetics of natural peptides (peptidomimetics).[1−27] The mode of covalent binding to the redox-active scaffold ferrocene remains the same in all these compounds, i.e., formation of an amide bond. Thus, the ferrocene moiety needs to be incorporated into the protecting group strategy for a given peptide, in both solution and solid phase synthesis.

In order to overcome these limitations, we searched for an orthogonal approach for the covalent binding of ferrocene to

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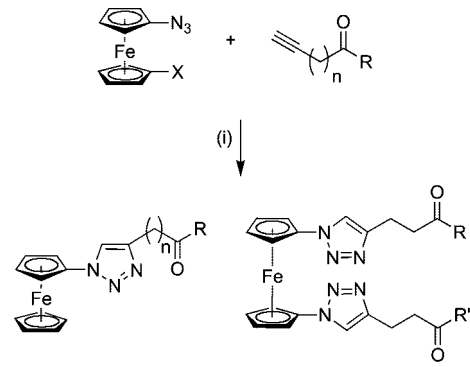
biomolecules. It has already been shown by our group that the Sonogashira coupling is an alternative procedure to label both the *N*- and *C*-terminus of peptides.[28−31] Although this method is versatile, as it tolerates functional groups, such as alcohols, thioethers, esters, and amines and is high yielding, the Sonogashira coupling step is somewhat sensitive to air and is usually carried out in organic solvents. Another method developed by Jaouen et al. proceeds via the formation of a pyrilium salt from lithioferrocene and 2,6-dimethyl-γ-pyrone, followed by acidification and reaction with amines to yield pyridinium salts.[32] The organometallic moiety is formed in low yields only, and to the best of our knowledge this method has not yet been used to label biomolecules. However, recently Sharpless et al. and Meldal et al. independently introduced the Cu(I)-catalyzed variant of the Huisgen [3+2] cycloaddition of azides and terminal alkynes (CuAAC) to form 1,2,3-triazoles, which is nowadays referred to as a so-called click reaction.[33,34] While the uncatalyzed, thermally driven reaction yields mixtures of 1,4- and 1,5-disubstituted triazoles, CuAAC exclusively affords 1,4-disubstituted triazoles at room temperature in high yields. An important feature in the bioconjugation context is that the reaction can proceed in water and under mild conditions. Furthermore, the high chemoselectivity of the azide moiety toward alkynes is contrasted by the practical inertness to highly functionalized biomolecules, which together with the high stability of the triazole ring makes this click reaction an attractive candidate for orthogonal bioconjugation. Due to these numerous advantages, click chemistry has been widely used in the bioconjugation area. Two very recent bioorganic examples serve to demonstrate the scope of the reaction: Bertozzi et al. describe the copper-free *in vivo* click labeling of zebrafish embryos with fluorescent probes,[35] while Carrell and co-workers reported the multilabeling of DNA using click chemistry.[36] In contrast, much less effort has been devoted to the preparation of organometallic bioconjugates. Along those lines, Schibli et al. reported the "click-to-chelate" approach for the radiolabeling of biomolecules,[37] and Santoyo-González et al. the [3+2] cycloaddition of propargyl glycosides with azidomethylferrocene and bis(azidomethyl)ferrocene.[38] Again only very recently, our group demonstrated the feasibility of the synthesis of (multi)organometallic PNA oligomers by the click reaction of azidoferrocene and alkyne-modified PNA oligomers.[39]

Scheme 1. Synthesis of Ferrocene Triazoles 1−10 in Solution[a]



X = H (azidoferrocene) Alkyne = pentyne (**1**)
n = 0, R = Leu-OMe (**2**)
n = 2, R = Phe-OMe (**3**), Ala-OMe (**4**), Enk-OH (**9**)
X = N_3 (diazidoferrocene) n = 2, R = R' = Leu-OMe (**5**), Val-OtBu (**6**), Phe-OMe (**7**), Pro-OMe (**8**), Enk-OH (**10**)

[a] Conditions: (i) 1.5 mol % Cu(II)SO_4, 15 mol % sodium ascorbate, *t*-BuOH/H_2O (1:1, v/v), 24−48 h, N_2, RT.

In this paper, we report the Cu(I)-catalyzed [3+2] cycloaddition of azidoferrocene and 1,1′-diazidoferrocene with alkyne-modified amino acids and peptides to form 1,4-disubstituted 1,2,3-triazole linked bioconjugates. To the best of our knowledge, this is the first report on the direct synthesis of nonradioactive organometallic peptide bioconjugates by click chemistry.

Results and Discussion

Synthesis of Mono- and Disubstituted Ferrocenyl Triazoles 1−10. Following the synthetic procedure of Sharpless et al.,[33] the monosubstituted ferrocenyl triazoles **1**, **2**, **3**, **4**, and **9** and the disubstituted ferrocenyl triazoles **5**, **6**, **7**, **8**, and **10** were prepared by reacting azidoferrocene and 1,1′-diazidoferrocene with pentyne (**1**), alkyne-modified L-leucine methyl ester (**2**, **5**), L-phenylalanine methyl ester (**3**, **7**), L-alanine methyl ester (**4**), L-valine *tert*-butyl ester (**6**), L-proline methyl ester (**8**), and [Leu^5]-enkephalin (**9**, **10**) (Scheme 1).

Azidoferrocene and 1,1′-diazidoferrocene were prepared by successive lithiation, bromination, and azidation of ferrocene.[40,41] The alkyne-modified amino acids were prepared by generating the free amine from the hydrochloric salt by adding triethylamine to a THF solution of the corresponding amino acid. Subsequent addition of propionic acid (**2**) or 4-pentynoic acid (**3**−**8**), *N*-methylmorpholine, and isobutylchloroformiate gave the alkyne-modified amino acids in good yields.[28,29] For the preparation of [Leu^5]-enkephalin, Fmoc SPPS (solid phase peptide synthesis) was employed, sequentially adding the respective α-L-amino acid to the preloaded Leu-Wang resin ending with 4-pentynoic acid as the terminal group. Cleavage from the solid support was performed in trifluoroacetic acid (TFA)/triisopropylsilane (TIS)/water, 95:2.5:2.5 (v/v/v). The alkyne moiety was activated by *in situ* formation of Cu(I) from a mixture of Cu(II)SO_4 and sodium ascorbate in a 1:1 water/*tert*-butanol mixture (v/v) and subsequent generation of Cu-acetylide. In a regioselective [3+2] cycloaddition of the azide and the alkyne moieties, the 1,4-disubstituted ferrocenyl triazoles were formed. All cycloaddition

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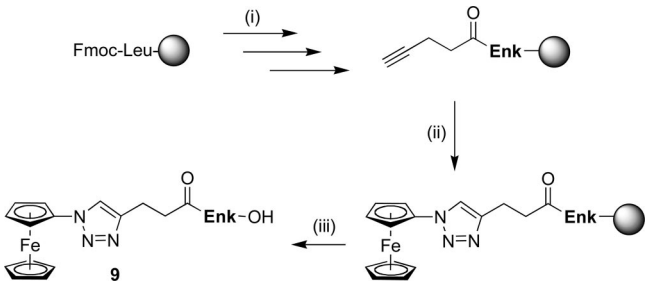
Scheme 2. Solid Phase Peptide Synthesis (SPPS) of Ferrocene Triazole 9[a]



[a] (i) Standard solid phase peptide synthesis (SPPS); (ii) 4.65 equiv of FcN_3, 3 equiv of CuI, 150 equiv of diisopropylethylamine (DIPEA), 48 h, N_2, RT; (iii) cleavage with trifluoroacetic acid (TFA/phenol/triisopropylsilane (TIS) (85:10:5, v/v/v).

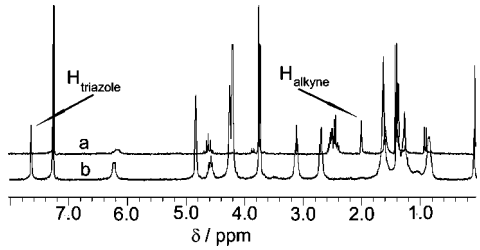


Figure 1. ^{1}H NMR spectra of (a) alkynyl precursor (25 °C, 250 MHz, $CDCl_3$) and (b) **4** (25 °C, 400 MHz, $CDCl_3$).

reactions were stirred for 24−48 h under inert conditions and exclusion of light to avoid oxidative alkyne−alkyne coupling and destruction of the azide. All products were purified by flash column chromatography on silica (**1**−**8**) or by reverse phase (RP)-HPLC (**9** and **10**) and could be isolated in good to excellent yields. The pure compounds were fully characterized by multinuclear and two-dimensional NMR spectroscopy, IR spectroscopy, and GC-, FAB-, and ESI-mass spectrometry (see Experimental Section and Supporting Information for full details).

Solid Phase Synthesis of Ferrocenyl Triazole 9. In addition to the reactions in solution, compound **9** was prepared on the solid phase (Scheme 2). In this case however, reaction conditions had to be somewhat modified because of the insolubility of sodium ascorbate and copper(II) sulfate in organic solvents and the undesired shrinking of the (Fmoc)-Leu Wang resin in aqueous solvent mixtures. Alkyne-modified [Leu^5]-enkephalin was prepared by Fmoc-SPPS as described above. On-resin click reaction with azidoferrocene was carried out in DMF with CuI as catalyst and a large excess of DIPEA (150 equiv) for better solubility of the Cu(I) salt.[34,39] The mixture was shaken for 2 days under a nitrogen atmosphere, after which cleavage with TFA/Phenol/TIS, 85:10:5 (v/v/v), afforded the product in 91% crude yield. Analytical HPLC and ESI-MS show clean product formation, with no more alkyne starting material that could be detected.

Spectroscopic Characterization of Ferrocenyl Triazoles 1−10. All ^{1}H NMR spectra of the ferrocenyl triazoles show characteristic features of the formation of the desired click products. The alkyne-modified starting material is identified by a signal at ca. 2 ppm, which corresponds to the terminal proton of the alkyne. Upon formation of the triazole ring, this signal disappears and a new signal appears at ca. 8 ppm, corresponding to the triazole proton (Figure 1).

The presence of monosubstituted ferrocene (compounds **1**, **2**, **3**, **4**, and **9**) is proven by signals corresponding to the ferrocene protons at 4.8, 4.3, and 4.2 ppm in a 2:2:5 ratio and of disubstituted ferrocene (compounds **5**, **6**, **7**, **8**, and **10**) by signals corresponding to the ferrocene protons at 5.0 and 4.3 ppm in a 4:4 ratio. Taking a closer look at the proton signals of the Cp rings of **6** and **7**, a splitting of the signals from the ferrocene H_α and H_β protons in $CDCl_3$ was observed, whereas in hydrogen-bonding solvents such as DMSO-d_6 no splitting was noticed. This is also true for both **9** and **10**, which were measured only in DMSO-d_6 due to their limited solubility. Five distinct signals in the ^{13}C NMR spectra of **5**, **6**, and **7** indicate magnetically nonequivalent carbon atoms of the Cp rings, while the ^{13}C NMR spectra of the monosubstituted reference compounds **3** and **4** exhibit only three signals for the substituted Cp ring, rendering the two ferrocene C_α as well as the C_β atoms magnetically equivalent (see Supporting Information for details). This would support intramolecular hydrogen bonds (IHBs) in **5**, **6**, and **7**, as the existence of diastereotopic protons and carbon atoms in the Cp rings cannot be attributed to the chirality of a triazole-linked amino acid alone.[7] Concentration-dependent ^{1}H NMR spectra of **7** in the concentration range 1−63 mM show that the chemical shift of the amide protons is only weakly dependent on the concentration. Consequently, intermolecular hydrogen bonds can be ruled out. Variable-temperature ^{1}H NMR spectra of **7** between 293 and 223 K show a downfield shift of the amide protons upon cooling with $\Delta\delta(\mathrm{NH}) = -10.8$ ppb K^{-1}, suggesting that the NH groups are involved in dynamic hydrogen bonding.[18] For the chemical shift of the triazole ring protons a minor temperature dependence with $\Delta\delta(\mathrm{CH}) = -4.1$ ppb K^{-1} is observed. This would speak for a weak intramolecular interaction of the triazole ring protons with the lone electron pairs of nitrogen of the second triazole ring, supporting the idea of IHBs in these compounds (see Supporting Information for details). To obtain a more quantitative view as to what extent the NH groups of **5**, **6**, and **7** are involved in IHBs, NH chemical shifts were measured in different solvents (variation ratio (vr) method).[25,43] For the assignment of δ_{NH} values it is useful to compare them to the NH shifts of reference compounds **3** and **4**, which cannot be involved in IHBs but have NH groups in a chemical environment similar to that of **5**, **6**, and **7**. Variation of the chemical shift from $CDCl_3$ to DMSO-d_6 ($\Delta\delta_{NH}$) provides a measure of the involvement in IHBs of a given amide proton; that is, if the variation is significantly smaller than that of the hydrogen-bonded free reference, the particular NH proton is thought to be involved in IHBs in $CDCl_3$ solution. This can be quantitatively assigned by the variation ratio $\mathrm{vr} = \Delta\delta_{NH}(\mathrm{sample})/\Delta\delta_{NH}(\mathrm{reference})$, where small vr values indicate strong hydrogen bonds, while weak ones will have large vr values. The vr values calculated for **5**, **6**, and **7** are in the range from 0.38 to 0.68 (see Supporting Information for details), indicating medium-strength IHBs in $CDCl_3$ solution,[7,25] which are disrupted in coordinating solvents such as DMSO as shown by CD spectroscopy (see below).

UV−vis spectra of ferrocene triazoles **1**−**9** showed the characteristic d−d bond transition of ferrocene at about 437 nm in CH_2Cl_2 and in a CH_2Cl_2/DMSO mixture (20% DMSO v/v). However compared to literature values, a hypsochromic shift of λ_{max} of about 10 nm is observed. Compound **10** was measured in pure DMSO due to its limited solubility and shows an absorption maximum at 405 nm.

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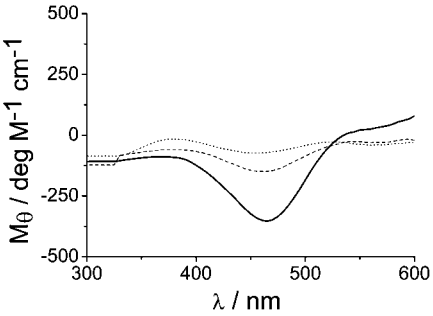

Figure 2. CD spectra of **3** (···), **7** (—), and **7** + DMSO (20% v/v) (---) in CH_2Cl_2 (2 mM) at 20 °C.

CD spectroscopy shows negative Cotton effects for the compounds **5**, **6**, and **7** at 460 to 465 nm in CH_2Cl_2 and at 449 nm for compound **10** in DMSO, indicative of a chiral environment around the ferrocene core (see Supporting Information for details). As expected, no distinct Cotton effect was observed for **8**, which does not have any amide protons. The CD maxima are shifted to lower energies compared to the UV-vis maxima, which is due to the electronic splitting of the ferrocene absorption band.[42] The observed negative Cotton effect suggests an *M*-helical arrangement of the ferrocene moiety as previously shown by Hirao,[11] Kraatz,[45] Heinze and Rapić,[43] and our group.[7,25] This is a remarkable observation, as it has been reported by Hirao[12] and Beck[44] that a helically ordered ferrocene moiety will only prevail when the amino acids or peptides form amide bonds directly attached to the ferrocene Cp rings. Furthermore, the *M*-helical ferrocene conformation has previously been attributed to IHBs of D-amino acids in conjugates with ferrocene dicarboxylic acid.[7,11,25,45] Obviously, the triazole ring provides sufficient rigidity for a stable helical conformation at the ferrocene core. At the same time, the greater distance between the chiral L-amino acids and the ferrocene core inverts the usually observed *P*-helical conformation to an *M* helix. Alternatively, the orientation of the triazole rings may also play a role in determining the symmetry of the compound and thus the observed CD spectrosocpic properties. If IHBs contribute to the observed conformation in CH_2Cl_2 solution, a decrease of the intensity of the CD signal would be expected in coordinating solvents, such as DMSO, that compete for hydrogen-bonding sites and thus disrupt IHBs.[18] In line with this reasoning, the intensity of the CD signals decreased by a factor of 2.6 (**5**), 2.3 (**7**), and 1.5 (**6**) upon addition of DMSO to CH_2Cl_2 solutions of **5**, **6**, and **7** (Figure 2).

Electrochemistry. Cyclic voltammetry (CV) of compounds **1**−**7**, **9**, and **10** was performed in CH_3CN at scan rates of 10−1000 mV/s with decamethylferrocene (Cp^*_2Fe) as an internal reference. Cp^*_2Fe was used instead of ferrocene (FcH) to avoid a possible overlap of the oxidation processes of $FcH^{0/+}$ and Fc-triazole$^{0/+}$.[46,47] Indeed, in most solvents, the potential of the $Cp^*_2Fe^{0/+}$ redox process is close to −500 mV vs $FcH^{0/+}$.[48] The monosubstituted compounds **1**−**4** and **9** show (quasi)reversible waves with formal potentials ΔE^0_f between +187 and +241 mV vs $FcH^{0/+}$ and the disubstituted compounds **5**−**7** with ΔE^0_f between +347 and +361 mV vs $Cp^*_2Fe^{0/+}$. Peak to peak separations ΔE_p of 62 to 121 mV are observed, with a Faradaic current ratio of close to unity (Figure 3, Table 1). Looking at the formal potentials ΔE^0_f of monosubstituted versus disubstituted ferrocenes, the higher electron-withdrawing character of two triazole moieties results in an increase of the oxidation potentials for the disubstituted compared to the monosubstituted triazoles. Within the group of monosubstituted triazoles an increase of approximately +50 mV in the oxidation potential of compound **2**, with a carbonyl group directly adjacent to the triazole, also shows an increased electron-withdrawing character compared to **1**, **3**, and **4**. Unlike all other compounds investigated in this study, compound **10** exhibits an irreversible oxidation with an E^{ox} = +282 mV vs $FcH^{0/+}$.

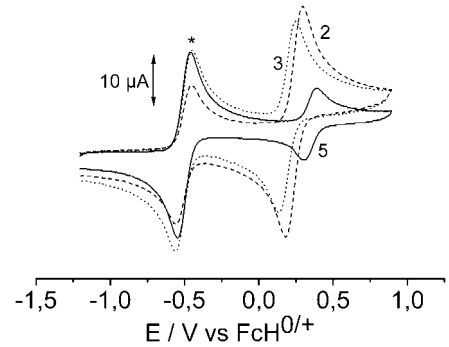

Figure 3. Cyclic voltammograms of **2** (1 mM), **3** (0.8 mM), and **5** (0.6 mM) in CH_3CN with Bu_4NPF_6 as the supporting electrolyte (0.1 M). The experiments were undertaken at 20 ± 2 °C with Cp^*_2Fe (*) as the internal reference (0.5 mM, at −505 mV vs FcH).

The diffusion coefficients (*D*) of **1**−**7** (Table 1) were calculated from the linear dependence of the peak current on the square root of the scan rate and the Randles−Sevcik equation.[49] Figure 4 shows the correlation of molecular mass and diffusion coefficient within the standard deviation; that is, the higher the molecular weight, the lower the diffusion coefficient.

Conclusion

It has been shown that 1,1′-diazidoferrocene forms symmetric 1,1′-disubstituted ferrocenyl triazoles with alkyne-modified α-amino acids and peptide [Leu^5]-enkephalin by Cu(I)-catalyzed [3+2] cycloaddition. Thorough NMR and CD spectroscopic studies show that medium strengh intramolecular hydrogen bonds exist in weakly coordinating solvents, inducing a *M*-helical arrangement on the ferrocene core. Reversible oxidation−reduction processes are found for all compounds but one (**10**) in cyclic voltammetry. The higher electron-withdrawing character of two triazole moieties results in an increase of the oxidation potentials for the disubstituted triazoles compared to the monosubstituted reference compounds.

Experimental Section

General Remarks. Reactions in solution were carried out under positive nitrogen pressure using standard Schlenk techniques. Azidoferrocene, 1,1′-diazidoferrocene, and alkyne-modified amino acids were prepared by published procedures;[28,29,40,41] all other chemicals were obtained commercially and used without further purification. Pure L amino acid methyl and *tert*-butyl esters were

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Table 1. Cyclic Voltammetry Data for Compounds 1−7, 9, and 10 in CH_3CN with Bu_4NPF_6 as the Supporting Electrolyte (0.1 M) (experiments were undertaken at 20 ± 2 °C with Cp^*_2Fe as the internal reference (0.5 mM))

compound	scan rate/mV s^{-1}	ΔE^0_f vs $Fc^{0/+}$/mV[a]	ΔE_p/mV[a]	$I_p^{ox}/\mu A$	$I_p^{red}/\mu A$	$I_p^{ox}/^bI_p^{red}$	$D/cm^2\ s^{-1}\ 10^{-5}$
1	250	191	111	30.2	32.2	0.94	4.1
2	250	241	112	24.4	23.2	1.05	3.0
3	250	196	112	19.0	18.4	1.03	2.8
4	250	192	103	17.2	15.6	1.10	2.5
5	250	347	104	11.6	9.8	1.18	1.2
6	250	361	121	9.2	8.6	1.07	0.4
7	250	343	101	10.6	9.22	1.15	0.01
9	250	187	63	2.0	2.0	1	–[d]
10	250	282[c]	–	0.6	–	–	–[d]

[a] E_p^{ox} = oxidation peak potential; E_p^{red} = reduction peak potential; formal redox potential $E_o^f = (E_p^{ox} + E_p^{red})/2$. [b] I_p^{ox} = oxidation peak current; I_p^{red} = reduction peak current; I_p^{ox}/I_p^{red} determined graphically. [c] Only E_p^{ox} due to irreversible oxidation. [d] No data due to poor solubility in CH_3CN.

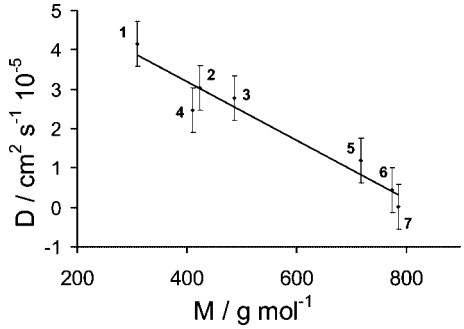

Figure 4. Correlation of molecular mass (*M*) and diffusion coefficient (*D*).

used. ^1H and $^{13}C\{^1H\}$ NMR spectra were recorded at room temperature on a Bruker DPX 400 (400 MHz) or DPX 250 (250 MHz) spectrometer. Chemical shifts, δ, are given in ppm relative to TMS and are referenced by using the residual undeuterated solvent signal. Coupling constants, *J*, are reported in Hz, multiplicities being marked as singlet (s), doublet (d), triplet (t), or multiplet (m). IR spectra were measured on a Bruker Tensor 27 with an ATR unit. Absorption bands are given in cm^{-1}, their intensities indicated as strong (s), medium (m), and weak (w). GC-MS spectra were recorded on a Shimadzu GCMS-OP2010. The retention time t_R is given in minutes. FAB-MS spectra were measured on a VG autospec; ESI-MS spectra, on a Bruker Daltonics Esquire 6000. The mass to charge relation (*m*/*z*) is given as a dimensionless number; peak intensity is given in percent relative to the base peak. HR-EI mass spectra were measured on a VG Autospec instrument with a resolution of 7000−8000. HR-ESI mass spectra were measured on a Thermo Scientific LTQ Orbitrap XL with a resolution of 60.000 running on Tune Plus software. Melting points were determined with a Büchi apparatus. UV/vis and CD spectra were recorded as 2 mM solutions in 1 cm Suprasil quartz cuvettes thermostated at 20 °C on a Varian Cary 100 spectrophotometer and a Jasco-715 spectropolarimeter, respectively. Absorption maxima, λ_{max}, are given in nm; molar absorption coefficients, ϵ, are given in $M^{-1}\ cm^{-1}$. Ellipticity maxima, λ_{max}, are given in nm. Molar ellipticity coefficients, M_θ, were calculated as $M_\theta = (100 \times \theta)/(c \times l)$, in which ellipticity θ is in degrees, concentration *c* is in mol L^{-1}, and path length *l* is in cm, to give units for M_θ of deg $mM^{-1}\ cm^{-1}$. HPLC was performed on a customized Varian ProStar 210 with PDA detector, column oven, and autosampler. A Varian DynaStar C-18 reversed phase column (250 × 8 mm) for analytical runs and (250 × 21 mm) for preparative runs was used. Mixtures of Millipore water and acetonitrile (Baker, HPLC grade) with 0.1% TFA (v/v) were used as eluents. Analytial measurements were done with a flow rate of 1.0 mL/min; semipreparative runs, with a flow rate of 4−5 mL/min. All chromatograms were recorded at λ = 220 and 254 nm at room temperature.

Electrochemical Studies. All voltammetric measurements were performed with a BES Princeton Applied Research potentiostat from Perkin-Elmer operated by Princeton Applied Research Software "Power Suite". A typical three-electrode cell was employed, comprising a Pt counter electrode, an $Ag/AgNO_3$ (acetonitrile, 10 mM $AgNO_3$) reference electrode, and a 2.0 mm diameter glassy carbon working electrode. In order to avoid overlap with the oxidation of the ferrocenyl pendant group, the voltammetrically reversible one-electron decamethylferrocene couple ($Cp^*_2Fe^{0/+}$) was used as a secondary internal reference (0.25 mM). Standard potentials were then referenced to the usual $FcH^{0/+}$ couple using measured values for the $Cp^*_2Fe^{0/+}$ process (−505 mV vs $FcH^{0/+}$ in acetonitrile as determined in the presence of Bu_4NPF_6 (0.1 M) as the supporting electrolyte at a scan rate of 100 $mV \cdot s^{-1}$).[47] It was assumed that these potential conversion values are independent of electrolyte and scan rate. The voltammetry for oxidation of ferrocene was used to calculate the area of the glassy carbon working electrode (0.023 cm^2) by using the Randles−Sevcik equation and the diffusion coefficient of ferrocene of 1.70×10^{-5} $cm^2 \cdot s^{-1}$ in acetonitrile (0.5 M $[NBu_4][PF_6]$).[49,50] All measurements were recorded over a scan rate range of 10 to 1000 $mV \cdot s^{-1}$ at 20 ± 2 °C inside a Faraday cage under an N_2 atmosphere to minimize electrochemical noise and atmospheric O_2/H_2O interference, respectively.

Synthesis of Mono- and Disubstituted Ferrocenyl Triazoles 1−8. Equimolar amounts of azidoferrocene and alkyne-Aaa-OMe/*t*Bu were placed in a Schlenk tube under a nitrogen atmosphere and suspended in 1 mL of a 1:1 mixture of water/*tert*-butanol. Fifteen mol % of a freshly prepared 1 M aqueous solution of sodium ascorbate and 1.5 mol % of $CuSO_4$ pentahydrate in water were added consecutively. The heterogeneous mixture was stirred vigorously for 24 h at RT under the exclusion of light. TLC analysis showed complete consumption of azidoferrocene. CH_2Cl_2 (10 mL) was added to the reaction mixture, and it was washed twice with water (2 × 10 mL), dried over Na_2SO_4, filtered, and evaporated under reduced pressure to yield a yellow-brown oil as crude product. Purification was carried out by flash column chromatography (silica, 0.062−0.2 mm, 5 g) using an ethyl acetate/*n*-hexane gradient.

$CpFe[C_5H_4\text{-}C_2N_3H\text{-}C_3H_7]$, 1: yellow-brown solid (71 mg, 70%); mp 86−88 °C; R_f = 0.84 (ethylacetate/hexane, 1:1). MS (FAB^+): *m*/*z* = 296.0 $[M + H]^+$ (100%), 318.0 $[M + Na]^+$ (32%). GC-MS (EI): t_R = 15.72 min, *m*/*z* = 295 $[M]^{+\bullet}$. HR-MS (EI): 295.0776 (calcd for $C_{15}H_{17}FeN_3$ 295.0772). ^1H NMR ($CDCl_3$, 400 MHz): δ 7.50 (s, 1H, $H_{Triazole}$), 4.81 (t, 2H, $H_{\alpha,Fc}$, ^3J = 2.0 Hz), 4.25 (t, 2H, $H_{\beta,Fc}$, ^3J = 2.0 Hz), 4.20 (s, 5H, H_{Fc}), 2.73 (t, 2H, $H_{\alpha,Pent}$, ^3J = 7.5 Hz), 1.75 (sept, 2H, $H_{\beta,Pent}$, ^3J = 7.5 Hz), 1.01 (t, 2H, $H_{\gamma,Pent}$, ^3J = 7.5 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 148.1 ($C_{i,Triazole}$), 120.1 ($C_{\alpha,Triazole}$), 93.9 ($C_{i,Fc}$), 69.9 (C_{Fc}), 66.3 ($C_{\alpha,Fc}$), 61.8 ($C_{\beta,Fc}$), 27.5 ($C_{\alpha,Pent}$), 22.5 ($C_{\beta,Pent}$), 13.6 ($C_{\gamma,Pent}$). Major IR bands (solid, cm^{-1}): 3123 (w), 3087 (w), 2955 (w), 2870 (w), 1550 (m), 1519 (w), 1466 (w), 1411 (w), 1377 (w), 1219 (m), 1106 (m), 1073 (w), 1041 (s), 1019 (m), 1001 (m), 877 (m), 815 (s), 738 (w).

$CpFe[C_5H_4\text{-}C_2N_3H\text{-}CO\text{-}Leu\text{-}OMe]$, 2: yellow-brown solid (65.5 mg, 77%); mp 113−115 °C; R_f = 0.78 (ethylacetate/hexane, 1:1).

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MS (FAB$^+$): m/z = 424.1 [M]$^+$ (100%), 425.1 [M + H]$^+$ (28%), 447.0 [M + Na]$^+$. GC-MS (EI): t_R = 24.95 min, m/z = 424 [M]$^{+\bullet}$. HR-MS (EI): 424.1182 (calcd for $C_{20}H_{24}FeN_4O_3$ 424.1198). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.28 (s, 1H, $H_{Triazole}$), 7.45 (d, 1H, NH_{Leu}, ^3J = 8.6 Hz), 4.88−4.82 (m, 1H, $H_{\alpha,Leu}$), 4.85 (m, 2H, $H_{\alpha,Fc}$), 4.31 (t, 2H, $H_{\beta,Fc}$, ^3J = 2.0 Hz), 4.22 (s, 5H, H_{Fc}), 3.77 (s, 3H, H_{OCH3}), 1.80−1.70 (m, 3H, $H_{\beta,Leu}$, $H_{\gamma,Leu}$), 0.99 (t, 6H, $H_{\delta,Leu}$, ^3J = 5.6 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.8 (CO_{Ester}), 159.7 (CO_{Amide}), 142.7 ($C_{i,Triazole}$), 124.9 ($C_{\alpha,Triazole}$), 93.1 ($C_{i,Fc}$), 70.3 (C_{Fc}), 67.1 ($C_{\alpha,Fc}$), 62.3 ($C_{\beta,Fc}$), 52.4 (C_{OCH3}), 50.5 ($C_{\alpha,Leu}$), 41.6 ($C_{\beta,Leu}$), 24.9 ($C_{\gamma,Leu}$), 22.9 ($C_{\delta,Leu}$), 21.8 ($C_{\delta,Leu}$). Major IR bands (solid, cm^{-1}): 3362 (w), 3120 (w), 3089 (w), 2957 (m), 2872 (w), 1743 (s), 1661 (s), 1566 (s), 1489 (s), 1434 (m), 1413 (m), 1360 (w), 1270 (m), 1225 (s), 1203 (s), 1189 (s), 1168 (s), 1149 (s), 1108 (m), 1036 (s), 1004 (m), 981 (w), 875 (m), 843 (m), 811 (s), 772 (m).

CpFe[C_5H_4-C_2N_3H-$(CH_2)_2$-CO-Phe-OMe], 3: yellow-brown solid (77 mg, 72%); mp 79−82 °C; R_f = 0.36 (ethylacetate/hexane, 2:1). Anal. Calcd for $C_{25}H_{26}N_4O_3Fe$ (486.3 g/mol): C, 61.74; H, 5.39; N, 11.52. Found: C, 61.83; H, 5.14; N, 11.42. MS (FAB$^+$): m/z = 487.1 [M + H]$^+$ (100%), 509.1 [M + Na]$^+$ (24%). GC-MS (EI): t_R = 27.58 min, m/z = 486 [M]$^{+\bullet}$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.59 (s, 1H, $H_{Triazole}$), 7.28−7.21 (m, 3H, $H_{Phe,m\ and\ p}$), 7.03 (d, 2H, $H_{phe,o}$, ^3J = 6.6 Hz), 6.06 (d, 1H, NH_{Phe}, ^3J = 7.6 Hz), 4.86 (ddd, 1H, $H_{\alpha,Phe}$, $^3J_1$ = 7.7 Hz, $^3J_2$ = 6.0 Hz, $^3J_3$ = 13.7 Hz), 4.81−4.80 (m, 2H, $H_{\alpha,Fc}$), 4.24 (t, 2H, $H_{\beta,Fc}$, ^3J = 2.0 Hz), 4.19 (s, 5H, H_{Fc}), 3.70 (s, 3H, H_{OCH3}), 3.06 (dd, 2H, $H_{\beta,Phe}$, $^3J_1$ = 5.8 Hz, $^3J_2$ = 13.9 Hz), 3.06 (dd, 2H, $H_{\beta,Pent}$, $^3J_1$ = 6.6 Hz, $^3J_2$ = 7.3 Hz), 2.65 (dd, 2H, $H_{\alpha,Pent}$, $^3J_1$ = 6.9 Hz, $^3J_2$ = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.7 (CO_{Ester}), 171.2 (CO_{Amide}), 146.1 ($C_{i,Triazole}$), 135.5 ($C_{i,Phe}$), 129.0 ($C_{m,Phe}$), 128.4 ($C_{o,Phe}$), 127.0 ($C_{p,Phe}$), 121.0 ($C_{\alpha,Triazole}$), 93.7 ($C_{i,Fc}$), 69.9 (C_{Fc}), 66.3 ($C_{\alpha,Fc}$), 61.8 ($C_{\beta,Fc}$), 53.0 ($C_{\alpha,Phe}$), 52.1 (C_{OCH3}), 37.7 ($C_{\beta,Phe}$), 35.3 ($C_{\alpha,Pent}$), 21.0 ($C_{\beta,Pent}$). Major IR bands (solid, cm^{-1}): 3386 (w), 3094 (w), 2926 (w), 1752 (s), 1650 (s), 1512 (s), 1442 (m), 1356 (m), 1275 (m), 1230 (m), 1209 (s), 1128 (m), 1107 (m), 1078 (w), 1045 (s), 1000 (m), 878 (m), 821 (s), 745 (m), 703 (s), 649 (w), 617 (w).

CpFe[C_5H_4-C_2N_3H-$(CH_2)_2$-CO-Ala-OMe], 4: yellow-brown solid (78 mg, 87%); mp 108−110 °C; R_f = 0.19 (ethylacetate/hexane, 2:1). Anal. Calcd for $C_{19}H_{22}N_4O_3Fe$ (410.2 g/mol): C, 55.63; H, 5.41; N, 13.66. Found: C, 56.17; H, 5.43; N, 13.20. MS (FAB$^+$): m/z = 411.1 [M + H]$^+$ (23%), 433.1 [M + Na]$^+$ (6%). GC-MS (EI): t_R = 23.0 min, m/z = 410 [M]$^{+\bullet}$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.63 (s, 1H, $H_{Triazole}$), 6.25 (d, 1H, NH_{Ala}, ^3J = 5.7 Hz), 4.81 (m, 2H, $H_{\alpha,Fc}$), 4.63−4.49 (m, 1H, $H_{\alpha,Ala}$), 4.24 (m, 2H, $H_{\beta,Fc}$), 4.19 (s, 5H, H_{Fc}), 3.72 (s, 3H, H_{OCH3}), 3.10 (t, 2H, $H_{\beta,Pent}$, ^3J = 7.0 Hz), 2.67 (t, 2H, $H_{\alpha,Pent}$, ^3J = 7.0 Hz), 1.37 (d, 3H, $H_{\beta,Ala}$, ^3J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.3 (CO_{Ester}), 171.5 (CO_{Amide}), 146.3 ($C_{i,Triazole}$), 121.3 ($C_{\alpha,Triazole}$), 93.9 ($C_{i,Fc}$), 70.1 (C_{Fc}), 66.6 ($C_{\alpha,Fc}$), 62.0 ($C_{\beta,Fc}$), 52.4 (C_{OCH3}), 48.1 ($C_{\alpha,Ala}$), 35.5 ($C_{\alpha,Pent}$), 21.3 ($C_{\beta,Pent}$), 18.3 ($C_{\beta,Ala}$). Major IR bands (solid, cm^{-1}): 3284 (w), 3090 (w), 2922 (w), 1745 (s), 1660 (s), 1548 (s), 1518 (m), 1441 (m), 1370 (w), 1344 (w), 1214 (s), 1158 (s), 1105 (m), 1045 (s), 1000 (m), 880 (w), 850 (w), 816 (s), 655 (w).

Fe[C_5H_4-C_2N_3H-$(CH_2)_2$-CO-Leu-OMe]$_2$, 5: yellow-brown solid (74 mg, 92%); mp 95−96 °C; R_f = 0.18 (ethylacetate). MS (FAB$^+$): m/z = 719.4 [M + H]$^+$ (40%), 741.4 [M + Na]$^+$ (60%). HR-MS (ESI$^+$): 719.2971 (calcd for $(C_{34}H_{46}FeN_8O_6$ + H) 719.2968). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.89 (s, 2H, $H_{Triazolee}$), 7.35 (d, 2H, NH_{Leu}, ^3J = 6.2), 5.07 (m, 4H, $H_{\alpha,Fc}$), 4.51−4.45 (m, 2H, $H_{\alpha,Leu}$), 4.31 (m, 4H, $H_{\beta,Fc}$), 3.80 (s, 6H, H_{OCH3}), 3.10−2.95 (m, 4H, $H_{\beta,Pent}$), 2.61−2.58 (m, 4H, $H_{\alpha,Pent}$), 1.89−1.77 (m, 4H, $H_{\beta,Leu}$), 1.74−1.68 (m, 2H, $H_{\gamma,Leu}$), 0.96−0.93 (t, 12H, $H_{\delta,Leu}$, ^3J = 5.2 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 174.2 (CO_{Ester}), 172.7 (CO_{Amide}), 145.9 ($C_{i,Triazole}$), 121.6 ($C_{\alpha,Triazole}$), 95.7 ($C_{i,Fc}$), 67.5 ($C_{\alpha,Fc}$), 67.3 ($C_{\alpha,Fc}$), 62.5 ($C_{\beta,Fc}$), 62.3 ($C_{\beta,Fc}$), 52.1 (C_{OCH3}), 51.7 ($C_{\alpha,Leu}$), 40.4 ($C_{\beta,Leu}$), 35.4 ($C_{\alpha,Pent}$), 29.7 ($C_{\beta,Pent}$), 24.9 ($C_{\delta,Leu}$), 22.9 ($C_{\delta,Leu}$), 22.1 ($C_{\gamma,Leu}$), 21.7 ($C_{\gamma,Leu}$). Major IR bands (solid, cm^{-1}): 3297 (w), 3093 (w), 2957 (m), 2870 (w), 2361 (w), 1740 (s) 1648 (s), 1521 (s), 1436 (m), 1369 (m), 1209 (s), 1160 (s), 1075 (w), 1045 (m), 1027 (m), 942 (w), 883 (m), 820(m), 651 (w).

Fe[C_5H_4-C_2N_3H-$(CH_2)_2$-CO-Val-O*t*Bu]$_2$, 6: yellow solid (77 mg, 89%); mp 122−124 °C; R_f = 0.24 (ethylacetate). MS (FAB$^+$): m/z = 775.4 [M + H]$^+$ (72%), 797.4 [M + Na]$^+$ (49%). HR-MS (ESI$^+$): 775.3587 (calcd for $(C_{38}H_{54}FeN_8O_6$ + H) 775.3594). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.63 (s, 2H, $H_{Triazole}$), 6.58 (d, 2H, NH_{Val}, ^3J = 8.4 Hz), 4.99−4.97 (m, 2H, $H_{\alpha,Fc}$), 4.91−4.90 (m, 2H, $H_{\alpha,Fc}$), 4.43−4.40 (dd, 2H, $H_{\alpha,Val}$, $^3J_1$ = 5.0 Hz, $^3J_2$ = 8.4 Hz), 4.29−4.28 (m, 4H, $H_{\beta,Fc}$), 3.07−3.03 (m, 4H, $H_{\alpha,Pent}$), 2.69−2.66 (m, 4H, $H_{\beta,Pent}$), 2.23 −2.18 (dd, 2H, $H_{\beta,Val}$, $^3J_1$ = 5.0 Hz, $^3J_2$ = 6.8 Hz), 1.48 (s, 18H, H_{OtBu}), 0.96−0.94 (d, 12H, $H_{\gamma,Val}$, ^3J = 6.8 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.2 (CO_{Ester}), 171.4 (CO_{Amide}), 146.6 ($C_{i,Triazole}$), 121.0 ($C_{\alpha,Triazole}$), 95.3 ($C_{i,Fc}$), 81.7 (C_{OtBu}), 67.8 ($C_{\alpha,Fc}$), 67.8 ($C_{\alpha,Fc}$), 63.1 ($C_{\beta,Fc}$), 62.8 ($C_{\beta,Fc}$), 58.0 ($C_{\alpha,Val}$), 35.3 ($C_{\alpha,Pent}$), 30.9 ($C_{\beta,Val}$), 28.1 (C_{OtBu}), 21.7 ($C_{\beta,Pent}$), 19.1 ($C_{\gamma,Val}$), 17.9 ($C_{\gamma,Val}$). Major IR bands (solid, cm^{-1}): 3333 (w), 3131 (w), 2963 (m), 2931 (s), 2361 (m), 2341 (m), 1731 (s), 1651 (s), 1519 (s), 1453 (m), 1390 (m), 1370 (s), 1332 (m), 1312 (m), 1259 (m), 1220 (s), 1147 (s), 1075 (m), 1044 (s), 1027 (m), 885 (m), 846 (m), 817 (s), 743 (w), 648 (w), 621 (w).

Fe[C_5H_4-C_2N_3H-$(CH_2)_2$-CO-Phe-OMe]$_2$, 7: brown solid (78 mg, 89%); mp 106−107 °C; R_f = 0.19 (ethylacetate). M_r($C_{40}H_{42}FeN_8O_6$) = 786.3. MS (FAB$^+$): m/z = 787.3 [M + H]$^+$ (34%), 809.3 [M + Na]$^+$ (43%). HR-MS (ESI$^+$): 787.2660 (calcd for $(C_{40}H_{42}FeN_8O_6$ + H) 787.2655). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.67 (s, 2H, $H_{Triazole}$), 7.30−7.19 (m, 10H, H_{Phe}), 7.13 (d, 2H, NH_{Phe}, ^3J = 6.9 Hz), 5.06−5.03 (m, 2H, $H_{\alpha,Fc}$), 5.02−5.01 (m, 2H, $H_{\alpha,Fc}$), 4.79−4.73 (dd, 2H, $H_{\alpha,Phe}$, $^3J_1$ = 7.5 Hz, $^3J_2$=6.0 Hz), 4.33−4.32 (t, 4H, $H_{\beta,Fc}$, ^3J = 2.0 Hz), 3.75 (s, 6H, H_{OCH3}), 3.25−3.12 (m, 4H, $H_{\beta,Phe}$), 3.07−2.91 (m, 4H, $H_{\beta,Pent}$), 2.56−2.52 (m, 4H, $H_{\alpha,Pent}$). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.6 (CO_{Ester}), 172.3 (CO_{Amide}), 146.1 ($C_{i,Triazole}$), 136.4 ($C_{i,Phe}$), 129.2 ($C_{m,Phe}$), 128.5 ($C_{o,Phe}$), 127.0 ($C_{p,Phe}$), 121.3 ($C_{\alpha,Triazole}$), 95.5 ($C_{i,Fc}$), 67.7 ($C_{\alpha,Fc}$), 67.6 ($C_{\alpha,Fc}$), 62.8 ($C_{\beta,Fc}$), 62.6 ($C_{\beta,Fc}$), 54.3 ($C_{\alpha,Phe}$), 52.2 (C_{OCH3}), 37.5 ($C_{\beta,Phe}$), 35.3 ($C_{\alpha,Pent}$), 21.7 ($C_{\beta,Pent}$). Major IR bands (solid, cm^{-1}): 3306 (w), 3089 (w), 2951 (w), 2360 (w), 1735 (s), 1644 (s), 1521 (s), 1435 (m), 1345 (m), 1278 (m), 1218 (s), 1178 (s), 1118 (w), 1078 (w), 1031 (m), 883 (w), 816 (m), 749 (m), 699 (s), 651 (m).

Fe[C_5H_4-C_2N_3H-$(CH_2)_2$-CO-Pro-OMe]$_2$, 8: brown solid (71 mg, 86%); R_f = 0.05 (ethylacetate). M_r($C_{32}H_{38}FeN_8O_6$) = 686.2; MS (FAB$^+$): m/z = 687.3 [M + H]$^+$ (12%), 709.2 [M + Na]$^+$ (24%). HR-MS (ESI$^+$): 687.2345 (calcd for $(C_{32}H_{38}FeN_8O_6$ + H) 687.2342). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.59 (s, 2H, $H_{Triazole}$), 4.94 (m, 2H, $H_{\alpha,Fc}$), 4.86 (m, 2H, $H_{\alpha,Fc}$), 4.50−4.48 (m, 2H, $H_{\alpha,Pro}$), 4.27 (m, 4H, $H_{\beta,Fc}$), 3.72 (s, 6H$_{OCH3}$), 3.59−3.45 (m, 4H, $H_{\delta,Pro}$), 3.13−3.03 (m, 4H, $H_{\beta,Pent}$), 2.80−2.73 (m, 4H, $H_{\alpha,Pent}$), 2.20−2.17 (m, 4H, $H_{\beta,Pro}$), 2.00−1.98 (m, 4H, $H_{\gamma,Pro}$). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.0 (CO_{Ester}), 170.8 (CO_{Amide}), 147.1 ($C_{i,Triazole}$), 121.4 ($C_{\alpha,Triazole}$), 95.2 ($C_{i,Fc}$), 68.4 ($C_{\alpha,Fc}$), 68.4 ($C_{\alpha,Fc}$), 63.3 ($C_{\beta,Fc}$), 63.2 ($C_{\beta,Fc}$), 58.7 ($C_{\alpha,Pro}$), 52.2 (C_{OCH3}), 47.0 ($C_{\delta,Pro}$), 33.4 ($C_{\alpha,Pent}$), 29.3 ($C_{\beta,Pro}$), 24.8 ($C_{\gamma,Pro}$), 20.7 ($C_{\beta,Pent}$). Major IR bands (solid, cm^{-1}): 3090 (w), 2923 (m), 2853 (m), 2360 (w),1737 (s), 1637 (s), 1519 (m), 1433 (s), 1365 (m), 1260 (m), 1197 (s), 1173 (s), 1096 (m), 1043 (s), 880 (m), 815 (s), 653 (m).

Synthesis of Monosubstituted Ferrocenyl Triazole 9 in Solution. Azidoferrocene (0.014 mmol, 3.2 mg) and alkyne-modified [Leu^5]-enkephalin (0.014 mmol, 8.9 mg) were suspended in 500 μL of a 1:1 mixture of water and *tert*-butanol. Then 2.1 μL (15 mol %) of a freshly prepared 1 M aqueous solution of sodium ascorbate and 2 μL (1.5 mol %) of a solution of 35 mg of $CuSO_4$ pentahydrate in 1.3 mL of water were added consecutively. The mixture was stirred for 24 h under a nitrogen atmosphere at RT. TLC of the reaction mixture in ethylacetate/hexane (1:1) showed

complete consumption of azidoferrocene. The reaction mixture was transferred to a falcon tube, the solvent evaporated to a few microliters, and the product precipitated with cold diethyl ether (−30 °C), centrifuged and washed with diethyl ether (2 ×), dissolved in CH_3CN/water (1:1), and lyophilized. Yield after preparative HPLC: 1.8 mg (15%).

Synthesis of 9 in the Solid Phase. The alkyne-modified peptide resin was weighed into a fritted syringe ("batch reactor") and swollen in DMF for 1 h. Azidoferrocene (4.65 equiv) and CuI (3 equiv) were put into the syringe from the top. A mixture of DIPEA (150 equiv) and DMF (100 μL per μmol resin) was taken up into the syringe, which was placed into a Schlenk tube and shaken for 48 h at room temperature under a nitrogen atmosphere and in the absence of light. The resin was then washed with DMF (5 ×) and CH_2Cl_2 (5 ×) and dried *in vacuo* for 1 h. Cleavage was carried out by adding TFA/phenol/TIS (85:10:5, v/v/v) to the resin, which was shaken for 2 h at room temperature. The cleavage solution was then evaporated to dryness, and the crude product precipitated with cold diethyl ether, centrifuged and washed with diethyl ether (2 ×), dissolved in CH_3CN/water (1:1), and lyophilized. Yield: 3.8 mg (91%) of a light yellow solid.

CpFe[C_5H_4-C_2N_3H-$(CH_2)_2$-CO-Enk-OH], 9: mp >120 °C (dec). $M_r(C_{43}H_{50}N_8O_8Fe)$ = 862.31. MS (ESI^+, MeOH): m/z = 863.23 $[M + H]^+$ (100%), 885.20 $[M + Na]^+$ (2%). MS (ESI^-, MeOH): m/z = 861.24 $[M - H]^-$ (100%). HR-MS (ESI^+): 863.3158 (calcd for $(C_{43}H_{50}FeN_8O_8 + H)$ 863.3179). t_R = 16.3 min. ^1H NMR (DMSO-d_6, 400 MHz): δ 9.15 (s, 1H, $H_{OH,Tyr}$), 8.27 (d, 1H, ^3J = 5.4 Hz, NH_{Gly}), 8.22 (d, 1H, ^3J = 7.6 Hz, N_{HLeu}), 8.16 (d, 1H, ^3J = 7.8 Hz, N_{HTyr}), 8.13 (s, 1H, $H_{Triazole}$), 7.98 (d, 1H, ^3J = 8.3 Hz, N_{HPhe}), 7.91 (d, 1H, ^3J = 5.8 Hz, N_{HGly}), 7.24−7.16 (m, 5H, $H_{Ar,Phe}$), 7.01 (m, 2H, $H_{Ar,Tyr}$), 6.62 (m, 2H, $H_{Ar,Tyr}$), 4.93 (m, 2H, $H_{α,Fc}$), 4.58−4.53 (m, 1H, $H_{α,Phe}$), 4.44−4.39 (m, 1H, $H_{α,Tyr}$), 4.30 (m, 2H, $H_{β,Fc}$), 4.24−4.20 (m, 1H, $H_{α,Leu}$), 4.18 (s, 5H, H_{Fc}), 3.69−3.58 (m, 4H, $H_{α,Gly}$), 2.81−2.78 (m, 2H, $H_{β,Tyr}$), 2.76−2.72 (m, 2H, $H_{β,Phe}$), 2.67−2.66 (m, 2H, $H_{β,Pent}$), 2.33−2.32 (m, 2H, $H_{α,Pent}$), 1.64−1.59 (m, 1H, $H_{γ,Leu}$), 1.56−1.50 (m, 2H, $H_{β,Leu}$), 0.86 (dd, 6H, ^3J = 5.2 Hz, ^2J = 21.8 Hz, $H_{δ,Leu}$).

Synthesis of Disubstituted Ferrocenyl Triazole 10 in Solution. 1,1′-Diazidoferrocene (3.73 μmol, 1.0 mg) and alkyne-modified [Leu^5]-enkephalin (7.71 mmol, 4.9 mg) were suspended in 500 μL of a 1:1 mixture of water and *tert*-butanol. Then 1.0 μL (10 mol %) of a freshly prepared 1 M aqueous solution of sodium ascorbate and 3.7 μL (5 mol %) of a solution of 6.6 mg of $CuSO_4$ pentahydrate in 245 μL of water were added consecutively. The mixture was stirred for 4 days under a nitrogen atmosphere at RT in the dark. *tert*-Butanol was evaporated, and the crude product was precipitated with cold diethyl ether, centrifuged and washed with ether (2 ×), dissolved in CH_3CN/water (1:1), and lyophilized.

Fe[C_5H_4-C_2N_3H-$(CH_2)_2$-CO-Enk-OH]$_2$, 10: yellow solid (4.5 mg, 79%); mp >120 °C (dec). $M_r(C_{76}H_{90}FeN_{16}O_{16})$ = 1538.6. MS (ESI^+, MeOH): m/z = 1539.2 $[M + H]^+$ (69%). MS (ESI^-, MeOH): m/z = 1537.3 $[M - H]^-$ (27%). t_R = 16.1 min. ^1H NMR (DMSO-d_6, 400 MHz): δ 11.23 (s, 2H, H_{COOH}), 9.12 (s, 2H, $H_{OH,Tyr}$), 8.27 (d, 2H, ^3J = 5.8 Hz, NH_{Gly}), 8.22 (d, 2H, ^3J = 7.9 Hz, N_{HLeu}), 8.14 (d, 2H, ^3J = 8.2 Hz, N_{HTyr}), 7.99 (d, 2H, ^3J = 8.4 Hz, N_{HPhe}), 7.96 (s, 2H, $H_{Triazole}$), 7.92 (d, 2H, ^3J = 5.7 Hz, N_{HGly}), 7.25−7.23 (m, 10H, $H_{Ar,Phe}$), 7.02 (d, 4H, ^3J = 8.4 Hz, $H_{Ar,Tyr}$), 6.63 (d, 4H, ^3J = 8.4 Hz, $H_{Ar,Tyr}$), 5.02 (m, 4H, $H_{α,Fc}$), 4.59−4.54 (m, 2H, $H_{α,Phe}$), 4.46−4.41 (m, 2H, $H_{α,Tyr}$), 4.31 (m, 4H, $H_{β,Fc}$), 4.25−4.19 (m, 2H, $H_{α,Leu}$), 3.75−3.63 (m, 8H, $H_{α,Gly}$), 2.79−2.76 (m, 4H, $H_{β,Tyr}$), 2.76−2.75 (m, 4H, $H_{β,Phe}$), 2.68−2.66 (m, 4H, $H_{β,Pent}$), 2.34−2.32 (m, 4H, $H_{α,Pent}$), 1.65−1.60 (m, 2H, $H_{γ,Leu}$), 1.56−1.51 (m, 4H, $H_{β,Leu}$), 0.86 (dd, 12H, ^3J = 6.4 Hz, ^2J = 21.4 Hz, $H_{δ,Leu}$).

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Supporting Information Available: ^1H and ^{13}C NMR spectra of compounds **3** and **7**, HPL chromatogram and ESI-MS spectra of **9** and **10**, variable concentration and temperature NMR plots of **7**, a table with variation ratio (vr) data of compounds **3**–**7** and a table with UV-Vis and CD data of **1**–**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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