Synthesis of Alkynyl Amino Acids and Peptides and Their Palladium-Catalyzed Coupling to Ferrocene

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A method for attaching organometallics to the C-terminus of amino acids via a Pd-catalyzed two-step procedure is presented. Boc-protected enantiomerically pure amino acids **1** (**a** Phe, **b** Leu, **c** Met, **d** Ser) are reacted with 1,1-diethylpropargylamine to yield alkynyl amino acids **2**. After reaction with (*p*-iodoanilido)ferrocene carboxylic acid **3** in the presence of 5 mol % $PdCl_2(PPh_3)_2$/CuI ferrocene amino acids **4** are obtained in ca. 80% yield. The reaction does not require anhydrous conditions and tolerates functional groups such as amides, alcohols (Ser, **4d**) or thioethers (Met, **4c**). A complete characterization by multinuclear NMR (including ^{15}N) is carried out. Cyclic voltammetry shows a reversible wave at about +190 mV (vs Fc/Fc^+) independent of the nature of the attached amino acid. In the solid state, **2a** forms a left-handed helix along the crystallographic *c* axis which is stabilized by hydrogen bonds as revealed by a single-crystal X-ray structure determination. A comparison of IR data in solution and the solid state suggests that hydrogen bonding is also important for the solid-state structures of ferrocene amino acids **4** but does not play a role in solution. The use of this methodology for peptide chemistry is demonstrated by labeling the dipeptide Boc-Met-Phe-OH at the C-terminus and the tripeptide Boc-Phe-Glu-Leu-OMe with ferrocene. The alkyne anchoring group in the tripeptide is introduced at the C_γ(Glu) atom at an early stage of the peptide synthesis and is not affected by subsequent deprotection and coupling reactions.

Introduction

Conjugates of biomolecules such as amino acids and peptides with covalently bound organometallic compounds have recently gained considerable attention.[1−11] In functional conjugates, the unique properties of the organometallic entity are exploited for the sensitive detection of the biomolecule.[12] For instance, a technique for monitoring the level of drugs such as phenobarbital in human blood using infrared spectroscopy has been developed for clinical applications in Jaouen's group and the name *Carbonylmetalloimmunoassay* (CMIA) has been coined.[2,13] In this assay, the standard procedure is the reaction of an activated organometallic acid with a primary amino group of the biomolecule like, for instance, phenobarbital. For the labeling of large biomolecules with a variety of functional groups its inherent unselectivity is one major disadvantage of this procedure. For example, in the labeling of bovine serum albumin (BSA) an average 40 of all 59 free amino groups (lysine and terminal NH_2) reacted with an activated $Co_2(CO)_6$ pentyne carboxylic acid derivative,[14] but only about 20 with a related Os compound.[15] Clearly, there is a need for the development of more selective methods of labeling.

In this work we present such a selective functionalization of amino acids and peptides with organometallics by making use of Pd-catalyzed coupling of terminal alkynes (as the anchoring group) with organometallic iodo-arenes. The advantage of our approach is the concept of *orthogonality*.[16,17] In organic synthesis and peptide chemistry, the term *orthogonality* is commonly used to indicate stability of one chemical moiety (e.g., a protecting group) under conditions where another group will easily react, usually giving rise to the selective, sequential functionalization of (organic) molecules. *Orthogonality* in the context of this work designates that (i) the reaction conditions used for binding the organometallic moiety leave most functional groups encountered in peptides untouched and (ii) the site of labeling can be unambiguously pre-selected by introducing the alkyne at the desired position. In the first step, a terminal alkyne is introduced at the site of labeling. Second, this alkyne is catalytically coupled

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(1) Severin, K.; Bergs, R.; Beck, W. *Angew. Chem.* **1998**, *110*, 1722−1743; *Angew. Chem., Int. Ed.* **1998**, *37*, 1634−1654.
(2) Jaouen, G.; Vessières, A.; Butler, I. S. *Acc. Chem. Res.* **1993**, *26*, 361−369.
(3) Ryabov, A. D. *Angew. Chem.* **1991**, *103*, 945−955; *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 931−941.
(4) Krämer, R. *Angew. Chem.* **1996**, *108*, 1287−1289; *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1197−1199.
(5) Sergheraert, C.; Brunet, J.-C.; Tartar, A. *J. Chem. Soc., Chem. Commun.* **1982**, 1417−1418.
(6) Sheldrick, W. S.; Gleichmann, A. J. *J. Organomet. Chem.* **1994**, *470*, 183−187.
(7) Gleichmann, A. J.; Wolff, J. M.; Sheldrick, W. S. *J. Chem. Soc., Dalton Trans.* **1995**, 1549−1554.
(8) Herrick, R. S.; Jarret, R. M.; Curran, T. P.; Dragoli, D. R.; Flaherty, M. B.; Lindyberg, S. E.; Slate, R. A.; Thornton, L. C. *Tetrahedron Lett.* **1996**, *30*, 5289−5292.
(9) Kraatz, H.-B.; Lusztyk, J.; Enright, G. D. *Inorg. Chem.* **1997**, *36*, 2400−2405.
(10) Kayser, B.; Polborn, K.; Steglich, W.; Beck, W. *Chem. Ber.* **1997**, *130*, 171−177.
(11) Ryabov, A. D.; Goral, V. N.; Gorton, L.; Csöregi, E. *Chem. Eur. J.* **1999**, *5*, 961−967.
(12) Salmain, M.; Vessières, A.; Brossier, P.; Butler, I. S.; Jaouen, G. *J. Immunol. Methods* **1992**, *148*, 65−75.
(13) Lavastre, I.; Besançon, J.; Brossier, P.; Moise, C. *Appl. Organomet. Chem.* **1990**, *4*, 9.
(14) Varenne, A.; Salmain, M.; Brisson, C.; Jaouen, G. *Bioconjugate Chem.* **1992**, *3*, 471−476.
(15) Osella, D.; Ravera, M.; Vincenti, M.; Salmain, M.; Jaouen, G. *Organometallics* **1996**, *15*, 3037−3041.
(16) Schelhaas, M.; Waldmann, H. *Angew. Chem.* **1996**, *108*, 2192−2219; *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2056−2083.
(17) Kocie'nski, R. *Protecting Groups*; G. Thieme Verlag: Stuttgart, 1994.

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to a suitably functionalized organometallic compound. During the course of this investigation, a related idea has been advanced by Schmidtchen and co-workers who employed a Pd-catalyzed coupling scheme for the detection of peptides by biotin−avidin technology.[18] In earlier work, modified nucleosides have been attached to ferrocene via ethenyl and ethynyl bridges,[19] and Beck et al. used a Heck coupling reaction with *p*-ethynylphenylalanine to assemble as many as four phenylalanine residues around one benzene ring.[20] Selective ferrocene functionalization of cysteine residues in peptides has been achieved by Di Gleria et al. using the thiol-specific *N*-(2-ferrocenylethyl)maleimide.[21] In this paper, the viability of our concept is first demonstrated for a number of different functional amino acids as model compounds which are coupled to a ferrocene derivative. The applicability of this concept for larger biomolecules is then demonstrated by labeling a di- and a tripeptide. Ferrocene has been chosen as a marker to exploit its potential in electrochemical detection.[22−24]

Experimental Section

All reactions were carried out in ordinary glassware and solvents without further precautions except where indicated. Chemicals were purchased from Aldrich-Sigma GmbH and Fluka AG and used as received, only enantiomerically pure L amino acids were used. Melting points (uncorrected) were determined in a Tottoli apparatus (Büchi, Switzerland). Elemental analyses were carried out by H. Kolbe, Analytisches Laboratorium, Mülheim. IR spectra were recorded on a Perkin-Elmer System 2000 instrument as KBr disks, additionally in CH_2Cl_2 solution where indicated. Frequencies ν are given in cm^{-1}. Raman spectra were recorded on the same instrument as neat substances (ν in cm^{-1}). UV/vis spectra were recorded on a Perkin-Elmer Lambda 19 spectrometer, only the wavelengths of the highest-energy ferrocene transitions are given in nm, ϵ in brackets. Mass spectra were recorded by the mass spectrometry service group, Mülheim, on a MAT 8200 (Finnigan GmbH, Bremen) instrument (EI, 70 eV) or on a MAT95 (Finnigan GmbH, Bremen) instrument (ESI, CH_3OH solution, positive ion detection mode). Only characteristic fragments from EI spectra are given with relative intensities (%) in brackets. Cyclic voltammogrammes were obtained with a three-electrode cell and an EG&G Princeton Applied Research model 273A potentiostat. A $Ag/AgNO_3$ (0.01 mol/L in $AgNO_3$) reference electrode, a glass carbon disk working electrode of 2 mm diameter and a Pt wire counter electrode was used. CH_2Cl_2 solutions (ca. 10^{-4} mol/L) contained 0.1 mol/L Bu_4NPF_6 as supporting electrolyte. As an internal standard, ferrocene was added in excess as a reference. NMR spectra were recorded in $CDCl_3$ at room temp. on Bruker ARX 250 (^{1}H at 250.13 MHz and ^{13}C), DRX 400 (^{1}H at 400.13 MHz, ^{13}C and 2D spectra) and DRX 500 (^{1}H at 500.13 MHz, ^{13}C, ^{15}N, 2D). ^{1}H and ^{13}C spectra were referenced to TMS, using the residual protio signals of the deuterated solvents as internal standards ($CDCl_3$: 7.24 ppm (^{1}H) and 77.0 ppm (^{13}C)). Positive chemical shift values δ (in ppm) indicate a downfield shift from the standard, only the absolute values of coupling constants are given in Hz. ^{15}N spectra were referenced to the absolute frequency of 50.696 991 0 MHz, which was the resonance frequency of neat nitromethane under the same experimental conditions. All resonances were assigned by 2D NMR (H−H−COSY and ^{1}H−^{13}C HMQC for ^1J and long-range couplings). ^{15}N chemical shifts and coupling constants were taken from the F1 projection of indirect detection ^{1}H−^{15}N correlated 2D spectra with 1024/256 data points in F1/F2, processed after applying a matched cosine function and zero filling in both dimensions. Mössbauer data were recorded on a spectrometer with alternating constant-accelaration and a ^{57}Co source in 6 μm Rh matrix. The minimum experimental line width was 0.24 mm s^{-1} full width at half-maximum. The sample temperature was maintained constant in an Oxford Instruments VARIOX cryostat. Isomer shifts are quoted relative to iron metal at 300 K.

X-ray Crystallographic Data Collection and Refinement. Crystal data for **2a**: $C_{21}H_{30}N_2O_3$, M = 358.47 $gmol^{-1}$, orthorhombic space group $P2_12_12_1$, a = 12.147(3) Å, b = 20.320(4) Å, c = 26.646(5) Å, U = 6577(2) Å^3, Z = 12, D_c = 1.086 Mg m^{-3}, μ(Mo Kα) = 0.072 mm^{-1}, F(000) = 2328. A transparent colorless single crystal of 1.12 × 0.49 × 0.35 mm^3 was sealed in a glass capillary and mounted on a Siemens SMART CCD-detector diffractometer system at ambient temperature. Graphite monochromated Mo Kα radiation (λ = 0.710 73 Å) was used. Cell constants were obtained from a least-squares fit of 6525 reflections. 22 426 intensities were collected by a hemisphere run taking frames at 0.30° in ω and corrected for Lorentz and polarization effects. The program SADABS (G. Sheldrick, University of Göttingen, Germany, 1994) was used for absorption correction. The Siemens ShelXTL software package (Siemens Analytical X-ray Instruments, Inc.) was used for solution and refinement of the structure. Neutral atom scattering factors were taken from the usual sources.[25] All non-hydrogen atoms were refined anisotropically. H-atoms were placed at calculated positions and refined as riding atoms with isotropic displacement parameters. The absolute structure could not be determined reliably but was assumed to be *L* since the stereochemistry was not influenced by our reactions. Final R1 = 0.0598, wR2 = 0.1281, 8355 unique reflections, 5960 reflections with $F_o > 4\sigma(F_o)$, 3.6° < 2θ < 45°, 704 parameters.

General Synthesis of Alkynyl Amino Acids 2a−d. Boc-protected L amino acid (5 mmol) was dissolved in 40 mL of thf at room temperature and neutralized with 5 mmol (0.51 g) of *N*-methylmorpholine. Isobutylchloroformate (5 mmol, 0.68 g) was added, and a white precipitate was rapidly formed. 1,1-Diethylpropargylamine (5 mmol, 0.56 g) was added, and the reaction mixture was stirred for 1 h. The hydrochloride of *N*-methylmorpholine was removed by filtration, and the solvent was removed on a rotary evaporator. The resulting residue was redissolved in ether, washed three times with water and dried over Na_2SO_4. After filtration the solvent was removed to yield 90−95% of white product. The product could be recrystallized from hot thf/heptane (1:20), which yielded single crystals suitable for an X-ray analysis in the case of **2a**.

2a. ^{1}H NMR: 7.26−7.18 (mult., 5H, $\mathbf{H}_{Ph}$), 5.85 (br, 1H, N**H**), 5.03 (br, 1H, N_{Boc}**H**), 4.21 (mult., 1H, C_α**H**), 3.01 (app. d, 2H, Ph-C$\mathbf{H}_2$), 2.30 (s, 1H, C≡C**H**), 2.06−1.95 (mult., 2H, CH_3−C$\mathbf{H}_2$), 1.77−1.66 (mult., 2H, CH_3−C$\mathbf{H}_2$), 1.39 (s, 9H, $C_{Boc}\mathbf{H}_3$), 0.87−0.78 (overlapping t, 6H, C$\mathbf{H}_3$−CH_2). ^{13}C NMR: 169.9 (C=O), 155.4 (C_{Boc}=O), 136.7 ($C_{Ph,i}$), 129.4, 128.7 (C_{Ph}), 126.9 ($C_{Ph,\ p}$), 84.6 (**C**≡CH), 80.3 (**C**$(CH_3)_3$), 71.6 (C≡**C**H), 57.0 (**C**$(Et)_2$), 56.4 (C_α), 38.1 (C_β), 30.45, 30.37 (CH_3−**C**H_2), 28.2 (C(**C**$H_3)_3$), 8.44, 8.38 (**C**H_3−CH_2). ^{15}N NMR: −291 ($\mathbf{N}_{Boc}$), −256. IR: 3310 (m), 1680 (s), 1656 (s). Raman: 2114. MS: 358 (1), 330 (13), 302 (4), 57 (100). Mp: 115 °C. Anal. Calcd for $C_{21}H_{30}N_2O_3$ (358.48 g/mol): C, 70.4; H, 8.4; N, 7.8. Found: C, 70.4; H, 8.7; N, 7.8.

2b. ^{1}H NMR: 6.23 (br, 1H, N**H**), 4.92 (br, 1H, N_{Boc}**H**), 3.97 (mult., 1H, C_α**H**), 2.29 (s, 1H, C≡C**H**), 2.16−2.09 (mult., 2H, CH_3−C$\mathbf{H}_2$), 1.78−1.68 (mult., 2H, CH_3−C$\mathbf{H}_2$), 1.63−1.60 (mult., 2H, C_β**H** and C_γ**H**), 1.39 (s and mult., 10H, $C_{Boc}\mathbf{H}_3$ and C_β**H**), 0.94−0.87 (overlapping t, 12H, C$\mathbf{H}_3$−CH_2 and $C_\delta\mathbf{H}_3$). ^{13}C NMR: 171.4 (C=O), 155.7 (C_{Boc}=O), 84.9 (**C**≡CH), 80.1 (**C**$(CH_3)_3$), 71.5 (C≡**C**H), 57.2 (**C**$(Et)_2$), 53.4 (C_α), 40.2 (C_β), 30.5 (CH_3−**C**H_2), 28.2 (C(**C**$H_3)_3$), 24.7 (C_γ), 22.7, 22.1 (C_δ), 8.5 (**C**H_3−CH_2). ^{15}N NMR: −289 ($\mathbf{N}_{Boc}$), −258. IR: 3317 (m), 1686 (s), 1658 (s). Raman: 2115. MS: 325 (0.1), 296 (4), 268 (1), 130 (100). Mp: 133 °C. Anal. Calcd for $C_{18}H_{32}N_2O_3$ (324.46 g/mol): C, 66.6; H, 9.9; N, 8.6. Found: C, 66.4; H, 10.0; N, 8.7.

2c. ^{1}H NMR: 6.23 (br, 1H, N**H**), 5.12 (d, J = 8 Hz, 1H, N_{Boc}**H**), 4.14 (mult., 1H, C_α**H**), 2.55−2.52 (mult., 2H, C_γ**H**), 2.33 (s, 1H,

(18) Dibowski, H.; Schmidtchen, F. P. *Angew. Chem.* **1998**, *110*, 487−489; *Angew. Chem., Int. Ed.* **1998**, *37*, 476−478.
(19) Meunier, P.; Ouattara, I.; Gautheron, B.; Tirouflet, J.; Camboli, D.; Besançon, J. *Eur. J. Med. Chem.* **1991**, *26*, 351−362.
(20) Kayser, B.; Altman, J.; Beck, W. *Tetrahedron* **1997**, *53*, 2475−2484.
(21) Di Gleria, K.; Nickerson, D.; Hill, H. A. O.; Wong, L.-L.; Fülöp, V. *J. Am. Chem. Soc.* **1998**, *120*, 46−52.
(22) Beer, P. D. *Acc. Chem. Res.* **1998**, *31*, 71−80.
(23) Eckert, H.; Seidel, C. *Angew. Chem.* **1986**, *98*, 168−170; *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 159−161.
(24) Eckert, H.; Koller, M. *J. Liq. Chromatogr.* **1990**, *13*, 3399−3414.
(25) *International Tables for Crystallography*; Kynoch Press: Birmingham, 1974; Vol. 4.

C≡C**H**), 2.08 (s, 3H, SC**H**$_3$), 2.14−2.02 (mult., 2H, CH$_3$−C**H**$_2$), 1.91−1.77 (overlapping mult., 4H, CH$_3$−C**H**$_2$ and C$_\beta$**H**), 1.42 (s, 9H, C$_{Boc}$**H**$_3$), 0.98−0.91 (overlapping t, 6H, C**H**$_3$−CH$_2$). ^{13}C NMR: 170.3 (C═O), 155.7 (C$_{Boc}$═O), 84.7 (**C**≡CH), 80.2 (**C**(CH$_3$)$_3$), 71.7 (C≡**C**H), 57.2 (**C**(Et)$_2$), 53.8 (C$_\alpha$), 30.6 (C$_\beta$), 30.5 (C$_\gamma$), 30.3, 30.0 (CH$_3$−**C**H$_2$), 28.3 (C(**C**H$_3$)$_3$), 15.3 (S**C**H$_3$), 8.55, 8.53 (**C**H$_3$−CH$_2$). ^{15}N NMR: −291 (**N**$_{Boc}$), −257. IR: 3309 (m), 1684 (s), 1659 (s). Raman: 2112. MS: 342 (7), 314 (7), 286 (2), 57 (100). Mp: 87 °C. Anal. Calcd for $C_{17}H_{30}N_2O_3S$ (342.50 g/mol): C, 59.6; H, 8.8; N, 8.2. Found: C, 59.2; H, 8.7; N, 7.9.

2d. ^{1}H NMR: 6.82 (br, 1H, N**H**), 5.65 (d, J = 7.1 Hz, 1H, N$_{Boc}$**H**), 4.02 (mult., 1H, C$_\alpha$**H**), 3.93 (mult., 1H, C$_\beta$**H**), 3.61 (mult., 1H, C$_\beta$**H**), 2.33 (s, 1H, C≡C**H**), 2.06−2.00 (mult., 2H, CH$_3$−C**H**$_2$), 1.83−1.71 (mult., 2H, CH$_3$−C**H**$_2$), 1.41 (s, 9H, C$_{Boc}$**H**$_3$), 0.96−0.88 (overlapping t, 6H, C**H**$_3$−CH$_2$). ^{13}C NMR: 170.1 (C═O), 155.7 (C$_{Boc}$═O), 84.4 (**C**≡CH), 80.2 (**C**(CH$_3$)$_3$), 73.7 (C≡**C**H), 62.4 (C$_\beta$), 57.1 (**C**(Et)$_2$), 55.2 (C$_\alpha$), 30.3 (CH$_3$−**C**H$_2$), 28.1 (C(**C**H$_3$)$_3$), 8.3 (**C**H$_3$−CH$_2$). ^{15}N NMR: −295 (**N**$_{Boc}$), −255. IR: 3425 (m), 3313 (m), 1752 (s), 1702 (s, br). Raman: 2117. MS: 298 (0.1), 270 (7), 242 (2), 225 (5), 214 (39), 82 (68), 57 (100). **2d** was obtained as a sticky oil which could not be purified from trace impurities.

3. Ferrocene carboxylic acid (1.00 g, 4.35 mmol) and 0.83 g (6.5 mmol) oxalic acid in 60 mL of dry dichloromethane were heated to reflux under argon for 1 h. After cooling to room temperature the solvent and excess oxalic acid were removed in vacuo to yield the ferrocene carboxylic acid chloride, which was redissolved in 60 mL of dichloromethane. 4-Iodoaniline (0.95 g, 4.35 mmol) and 0.44 g (4.35 mmol) of triethylamine were slowly added at room temperature and the solution was allowed to stirr overnight. After removal of triethylamine hydrochloride the solvent was removed in vacuo. The residue was dissolved in chloroform, the organic phase washed three times with water and dried over Na_2SO_4. After filtration the solvent was removed to yield 1.68 g (90%) of **3**. If necessary crude **3** can be recrystallized from warm methanol to give dark orange crystals. ^{1}H NMR: 7.63 (pseudo-d, 2H, **H**$_{Ar}$), 7.36 (pseudo-d, 2H, **H**$_{Ar}$), 7.29 (br, 1H, N**H**), 4.75 (pseudo-t, 2H, **H**$_{Cp}$), 4.42 (pseudo-t, 2H, **H**$_{Cp}$), 4.24 (s, 5H, **H**$_{Cp}$). ^{13}C NMR: 168.0 (C═O), 138.0 (C(N)−**C**$_{Ar}$), 121.5 (C(I)−**C**$_{Ar}$), 121.4 (C$_{Ar}$N), 86.9 (**C**$_{Ar}$I), 76.0 (**C**$_{Cp,i}$), 71.1 (**C**$_{Cp,o/m}$), 69.9 (**C**$_{Cp}$), 68.3 (**C**$_{Cp,m/o}$). IR: 3294 (br, m), 1638 (s). UV: 443 (337). MS: 431 (100), 213 (79), 185 (35), 129 (25). CV: +189 mV. Mp: 198 °C. Anal. Calcd for $C_{17}H_{14}FeINO$ (431.06 g/mol): C, 47.4; H, 3.3; N, 3.3. Found: C, 47.2; H, 3.5; N, 3.0.

General Synthesis of Ferrocene Amino Acids 4. A 0.5 g (1.16 mmol) amount of **3**, 40 mg (0.06 mmol) of bis(triphenylphosphine)-palladium(II) dichloride, and 10 mg (0.06 mmol) of copper(I) iodide were dissolved at room temperature in a deareated mixture of 40 mL of thf and 10 mL of triethylamine; 1.1 mmol of the respective alkynyl amino acid **2a**−**d** in 40 mL of thf was added dropwise at room temperature, and the solution soon became darker. After complete additon of the alkynyl amino acid the reaction mixture was immediately heated to reflux under argon for 4 h. After cooling to room temperature, the dark suspension was filtered and the solvents were removed on a rotary evaporator. The resulting residue was redissolved in chloroform. After being washed with water three times, the organic phase was dried over Na_2SO_4 and filtered, and the solvent was removed on a rotary evaporator to yield ca. 80% of light orange product. Unreacted **3**, which is almost insoluble in ether, could be removed by dissolving the crude product in a small amount of ether, filtration, and evaporation of the solvent.

4a. ^{1}H NMR: 7.55 (pseudo-d, 2H, **H**$_{Ar}$), 7.45 (br, 1H, C$_{Ar}$-N**H**), 7.38 (pseudo-d, 2H, **H**$_{Ar}$), 7.26−7.19 (mult., 5H, **H**$_{Ph}$), 5.85 (br, 1H, N**H**), 5.00 (br, 1H, N$_{Boc}$**H**), 4.76 (pseudo-t, 2H, **H**$_{Cp}$), 4.09 (pseudo-t, 2H, **H**$_{Cp}$), 4.25 (mult., 1H, C$_\alpha$**H**), 4.23 (s, 5H, **H**$_{Cp}$), 3.05−3.01 (mult., 2H, Ph-C**H**$_2$), 2.16−2.11 (mult., 2H, CH$_3$−C**H**$_2$), 1.84−1.71 (mult., 2H, CH$_3$−C**H**$_2$), 1.40 (s, 9H, C$_{Boc}$**H**$_3$), 0.95−0.85 (overlapping t, 6H, C**H**$_3$−CH$_2$). ^{13}C NMR: 169.8 (C$_{Phe}$═O), 168.7 (C$_{C-Cp}$═O), 155.4 (C$_{Boc}$═O), 138.2 (C$_{Ar}$), 136.7 (C$_{Ph,i}$), 132.6 (C$_{Ar}$), 129.4 (C$_{Ph}$), 128.7 (C$_{Ph}$), 126.9 (C$_{Ph,p}$), 119.2 (C$_{Ar}$), 118.1 (C$_{Ar}$), 89.7 (C$_{Ar}$-**C**≡C), 83.6 (C$_{Ar}$-C≡**C**), 80.2 (**C**(CH$_3$)$_3$), 76.0 (**C**$_{Cp,i}$), 71.0 (**C**$_{Cp,o/m}$), 69.9 (**C**$_{Cp}$), 68.3 (**C**$_{Cp,m/o}$), 58.3 (**C**(Et)$_2$), 56.4 (C$_\alpha$), 38.2 (C$_\beta$), 30.75, 30.69 (CH$_3$-**C**H$_2$), 28.3 (C(**C**H$_3$)$_3$), 8.84, 8.80 (**C**H$_3$−CH$_2$). ^{15}N NMR: −291 (**N**$_{Boc}$), −256, −253 (C$_{Ar}$−**N**). IR (KBr): 3316 (br, m), 1690 (sh), 1665 (s), 1647 (s). IR (CH$_2$Cl$_2$): 3429 (m), 1711 (s), 1677 (s). Raman: 2229. UV: 446 (400). MS: 661 (100), 605 (2), 587 (13), 561 (19), 385 (24), 213 (53). CV: +193 mV. Mp: 96 °C. Anal. Calcd for $C_{38}H_{43}FeN_3O_4$ (661.62 g/mol): C, 69.0; H, 6.6; N, 6.4. Found: C, 68.5; H, 6.8; N, 6.2.

4b. ^{1}H NMR: 7.55 (pseudo-d, 2H, **H**$_{Ar}$), 7.40 (br, 1H, C$_{Ar}$-N**H**), 7.40 (pseudo-d, 2H, **H**$_{Ar}$), 6.17 (br, 1H, N**H**), 4.82 (br, 1H, N$_{Boc}$**H**), 4.75 (pseudo-t, 2H, **H**$_{Cp}$), 4.41 (pseudo-t, 2H, **H**$_{Cp}$), 4.23 (s, 5H, **H**$_{Cp}$), 3.99 (mult., 1H, C$_\alpha$**H**), 2.30−2.25 (mult., 2H, CH$_3$−C**H**$_2$), 1.84−1.80 (mult., 2H, CH$_3$−C**H**$_2$), 1.67−1.62 (mult., 2H, C$_\beta$**H** and C$_\gamma$**H**), 1.46 (mult., 1H, C$_\beta$**H**), 1.42 (s, 9H, C$_{Boc}$**H**$_3$), 1.04−0.97 (overlapping t, 6H, C**H**$_3$−CH$_2$), 0.94−0.90 (overlapping t, 6H, C$_\delta$**H**$_3$). ^{13}C NMR: 171.2 (C$_{Leu}$═O), 168.6 (C$_{C-Cp}$═O), 155.4 (C$_{Boc}$═O), 138.1 (C$_{Ar}$), 132.7 (C$_{Ar}$), 119.2 (C$_{Ar}$), 118.1 (C$_{Ar}$), 90.0 (C$_{Ar}$−**C**≡C), 83.5 (C$_{Ar}$−C≡**C**), 80.1 (**C**(CH$_3$)$_3$), 76.0 (**C**$_{Cp,i}$), 71.1 (**C**$_{Cp,o/m}$), 69.9 (**C**$_{Cp}$), 68.3 (**C**$_{Cp,m/o}$), 58.7 (**C**(Et)$_2$), 53.7 (C$_\alpha$), 40.9 (C$_\beta$), 30.8 (CH$_3$−**C**H$_2$), 28.3 (C(**C**H$_3$)$_3$), 24.8 (C$_\gamma$), 22.9, 22.1 (C$_\delta$), 9.0, 8.9 (**C**H$_3$−CH$_2$). ^{15}N NMR: −289 (**N**$_{Boc}$), −258, −254 (C$_{Ar}$−**N**). IR (KBr): 3416 (br, m), 3324 (br, m), 1710 (sh), 1655 (s). IR (CH$_2$Cl$_2$): 3431 (m), 1711 (sh), 1677 (s). Raman: 2231. UV: 443 (380). MS: 627 (100), 571 (3), 553 (83), 385 (29), 213 (93). CV: +195 mV. Mp: 162 °C. Anal. Calcd for $C_{35}H_{45}FeN_3O_4$ (627.61 g/mol): C, 67.0; H, 7.2; N, 6.7. Found: C, 66.8; H, 7.3; N, 6.6.

4c. ^{1}H NMR: 7.54 (pseudo-d, 2H, **H**$_{Ar}$), 7.45 (br, 1H, C$_{Ar}$−N**H**), 7.38 (pseudo-d, 2H, **H**$_{Ar}$), 6.26 (br, 1H, N**H**), 5.10 (br, 1H, N$_{Boc}$**H**), 4.75 (pseudo-t, 2H, **H**$_{Cp}$), 4.41 (pseudo-t, 2H, **H**$_{Cp}$), 4.23 (s, 5H, **H**$_{Cp}$), 4.22 (mult., 1H, C$_\alpha$**H**), 2.60−2.52 (mult., 2H, C$_\gamma$**H**), 2.28−2.20 (mult., 2H, CH$_3$−C**H**$_2$), 2.09 (s, 3H, SC**H**$_3$), 2.06 (mult., 1H, C$_\beta$**H**), 1.93−1.82 (overlapping mult., 3H, CH$_3$−C**H**$_2$ and C$_\beta$**H**), 1.42 (s, 9H, C$_{Boc}$**H**$_3$), 1.04−0.98 (overlapping t, 6H, C**H**$_3$−CH$_2$). ^{13}C NMR: 170.2 (C$_{Met}$═O), 168.7 (C$_{C-Cp}$═O), 155.7 (C$_{Boc}$═O), 138.2 (C$_{Ar}$), 132.6 (C$_{Ar}$), 119.2 (C$_{Ar}$), 118.0 (C$_{Ar}$), 89.7 (C$_{Ar}$−**C**≡C), 83.5 (C$_{Ar}$−C≡**C**), 80.0 (**C**(CH$_3$)$_3$), 76.0 (**C**$_{Cp,i}$), 71.0 (**C**$_{Cp,o/m}$), 69.9 (**C**$_{Cp}$), 68.3 (**C**$_{Cp,m/o}$), 58.5 (**C**(Et)$_2$), 53.7 (C$_\alpha$), 31.4 (C$_\gamma$), 30.9 (C$_\beta$), 30.8, 30.4 (CH$_3$−C**H**$_2$), 28.3 (C(**C**H$_3$)$_3$), 15.3 (S**C**H$_3$), 9.0, 8.9 (**C**H$_3$−CH$_2$). ^{15}N NMR: −290 (**N**$_{Boc}$), −256, −253 (C$_{Ar}$−**N**). IR (KBr): 3324 (m), 1699 (sh), 1654 (s). IR (CH$_2$Cl$_2$): 3429 (m), 1710 (sh), 1678 (s). Raman: 2224. UV: 444 (360). MS: 645 (86), 589 (4), 571 (43), 545 (42), 385 (27), 213 (100). CV: +189 mV. Mp: 95 °C. Anal. Calcd for $C_{34}H_{43}FeN_3O_4S$ (645.64 g/mol): C, 63.3; H, 6.7; N, 6.5. Found: C, 62.8; H, 6.7; N, 6.1.

4d. ^{1}H NMR: 7.54 (pseudo-d, 2H, **H**$_{Ar}$), 7.49 (br, 1H, C$_{Ar}$−N**H**), 7.38 (pseudo-d, 2H, **H**$_{Ar}$), 6.80 (br, 1H, N**H**), 5.59 (br, 1H, N$_{Boc}$**H**), 4.75 (pseudo-t, 2H, **H**$_{Cp}$), 4.41 (pseudo-t, 2H, **H**$_{Cp}$), 4.23 (s, 5H, **H**$_{Cp}$), 4.09 (mult., 1H, C$_\alpha$**H**), 3.62 (mult., 1H, C$_\beta$**H**), 3.03 (mult., 1H, C$_\beta$**H**), 2.25−2.16 (mult., 2H, CH$_3$−C**H**$_2$), 1.91−1.77 (mult., 2H, CH$_3$−C**H**$_2$), 1.44 (s, 9H, C$_{Boc}$**H**$_3$), 1.05−0.99 (overlapping t, 6H, C**H**$_3$−CH$_2$). ^{13}C NMR: 170.2 (C$_{Ser}$═O), 168.9 (C$_{C-Cp}$═O), 155.7 (C$_{Boc}$═O), 138.2 (C$_{Ar}$), 132.6 (C$_{Ar}$), 119.3 (C$_{Ar}$), 118.0 (C$_{Ar}$), 89.6 (C$_{Ar}$−**C**≡C), 83.6 (C$_{Ar}$−C≡**C**), 80.0 (**C**(CH$_3$)$_3$), 75.9 (**C**$_{Cp,i}$), 71.0 (**C**$_{Cp,o/m}$), 69.9 (**C**$_{Cp}$), 68.3 (**C**$_{Cp,m/o}$), 62.7 (C$_\beta$), 58.2 (**C**(Et)$_2$), 54.9 (C$_\alpha$), 30.90, 30.85 (CH$_3$−**C**H$_2$), 28.2 (C(**C**H$_3$)$_3$), 8.8 (**C**H$_3$−CH$_2$). ^{15}N NMR: −295 (**N**$_{Boc}$), −255, −253 (C$_{Ar}$−**N**). IR (KBr): 3422 (m), 3327 (m), 1702 (sh), 1648 (s). IR (CH$_2$Cl$_2$): 3430 (m), 1714 (m), 1676 (s), 1513 (s). Raman: 2240. UV: 446 (400). MS: 601 (77), 527 (24), 385 (26), 213 (83), 41 (100). CV: +192 mV. Mp: 80 °C. Anal. Calcd for $C_{32}H_{39}FeN_3O_5$ (601.52 g/mol): C, 63.9; H, 6.5; N, 7.0. Found: C, 64.1; H, 6.6; N, 6.8.

5. The dipeptide Boc-Met-Phe-OH (0.54 g, 1.42 mmol) was dissolved in 50 mL of thf. *N*-Methylmorpholine (145 mg, 1.42 mmol) was added to the clear solution, followed by isobutylchloroformate (194 mg, 1.42 mmol) upon which a white precipitate of *N*-methylmorpholine hydrochloride formed. After additon of 158 mg (1.42 mmol) of 1,1-diethylpropargylamin the reaction mixture was stirred for 60 min at room temperature and filtered, and all volatiles were removed on a rotary evaporator. The residue was taken up in water, and the aqueous phase extracted with ether (four times). The combined organic phases were washed with water, dried over Na_2SO_4, and filtered, and the solvent was removed in vacuo. The white residue was recrystalized from hot heptane/thf to yield 0.58 g (1.18 mmol, 83%) of pure **5**. ^{1}H NMR ($CDCl_3$): 7.29−7.20 (mult., 5H, H$_{Phe}$), 6.78 (br, 1H, **N**$_{phe}$H), 5.83 (br, 1H, N$_{DEPA}$**H**), 5.12 (br, 1H, N$_{Boc}$**H**), 4.54 (mult., 1H, C$_{\alpha,Phe}$**H**), 4.18 (br, 1H, C$_{\alpha,Met}$**H**), 3.03 (mult., 2H, C$_{\beta,Phe}$**H**$_2$), 2.46 (C$_{\gamma,Met}$**H**$_2$), 2.31 (s,

1H, C≡C**H**), 2.06 (s, 3H, S−C**H**$_3$), 1.98 (mult., 2H, C**H**$_2$CH$_3$), 1.84 (mult., 1H, C$_{\beta,\mathrm{Met}}$**H**), 1.71 (mult., 3H, C$_{\beta,\mathrm{Met}}$**H** and C**H**$_2$CH$_3$) 1.39 (s, 9H, C$_{\mathrm{Boc}}$**H**$_3$), 0.81 (overlapping t, 6H, CH$_2$C**H**$_3$). ^{13}C NMR ($CDCl_3$): 171.2 (**C**$_{\mathrm{Met}}$═O), 169.1 (**C**$_{\mathrm{Phe}}$═O), 155.0 (**C**$_{\mathrm{Boc}}$═O), 136.4, 129.4, 128.7, 127.1 (C$_{\mathrm{Phe}}$), 84.5 (**C**≡CH), 80.1 (**C**$_{\mathrm{Boc}}$(CH$_3$)$_3$), 71.7 (C≡**C**H), 54.8 (**C**$_{\alpha,\mathrm{Phe}}$ and **C**$_{\alpha,\mathrm{Met}}$), 37.9 (**C**$_{\beta,\mathrm{Phe}}$), 31.2 (**C**$_{\beta,\mathrm{Met}}$), 30.4 and 30.2 (**C**H$_2$CH$_3$), 30.1 (**C**$_{\gamma,\mathrm{Met}}$), 28.3 (C$_{\mathrm{Boc}}$(**C**H$_3$)$_3$) 15.3 (S−**C**H$_3$), 8.4 (CH$_2$**C**H$_3$). ^{15}N NMR ($CDCl_3$): −293 (**N**$_{\mathrm{Boc}}$), −262 (**N**$_{\mathrm{Phe}}$), −254 (**N**$_{\mathrm{DEPA}}$). IR: 3427 (sh), 3277 (br), 1686 (m), 1644 (s). Raman: 2112. MS: 489 (11), 415 (38), 359 (22), 120 (100), 104 (29), 57 (77). Mp: 191 °C. Anal. Calcd for $C_{26}H_{39}N_3O_4S$ (489.67 g/mol): C, 63.8; H, 8.0; N, 8.6. Found: C, 63.7; H, 8.0; N, 8.4.

6. A mixture of 40 mL of thf and 10 mL of NEt_3 was deaerated, 277 mg (0.64 mmol) of **3** was dissolved, and CuI (5 mg, 0.03 mmol) and $(Ph_3P)_2PdCl_2$ (20 mg, 0.03 mmol) were added. Under Ar, a solution of 300 mg (0.61 mmol) of **5**, dissolved in 40 mL of thf, was slowly added and the reaction mixture was heated to 80 °C for 4 h. After filtration through a bed of Celite all volatiles were removed, the residue taken up in $CHCl_3$ and water, the organic phase separated, washed with water three times, dried over Na_2SO_4, and filtered, and the solvent removed on a rotary evaporator. The ferrocene-labelled dipeptide **6** was purified by HPLC on a 250 × 20 mm^3 Nucleosil N-7-C_{18} column (No. 312014) using a 4:1 CH_3OH/H_2O solvent mixture with 4 mL/min flow on a Merck C 6200 pump. A Shimadzu SPD-6-AV detector was operated at 420 nm. ^{1}H NMR ($CDCl_3$): 7.83 (s, 1H, N$_{\mathrm{Aniline}}$**H**), 7.57 (pseudo-d, 2H, **H**$_{\mathrm{Aniline}}$), 7.34 (pseudo-d, 2H, **H**$_{\mathrm{Aniline}}$), 7.26−7.20 (mult., 5H, **H**$_{\mathrm{Phe}}$), 6.95 (br, 1H, **N**$_{\mathrm{Phe}}$H), 6.03 (br, 1H, N$_{\mathrm{DEPA}}$**H**), 5.30 (br, 1H, N$_{\mathrm{Boc}}$**H**), 4.80 (pseudo-t, 2H, **H**$_{\mathrm{Cp}}$), 4.63 (mult., 1H, C$_{\alpha,\mathrm{Phe}}$**H**), 4.36 (pseudo-t, 2H, **H**$_{\mathrm{Cp}}$), 4.20 (br, 6H, C$_{\alpha,\mathrm{Met}}$**H** and **H**$_{\mathrm{Cp}}$), 3.02 (mult., 2H, C$_{\beta,\mathrm{Phe}}$**H**$_2$), 2.41 (C$_{\gamma,\mathrm{Met}}$**H**$_2$), 2.04 (mult, 3H, C**H**$_2$CH$_3$ and C$_{\beta,\mathrm{Met}}$**H**), 2.00 (s, 3H, S−C**H**$_3$), 1.78 (mult, 3H, C**H**$_2$CH$_3$ and C$_{\beta,\mathrm{Met}}$**H**), 1.37 (s, 9H, C$_{\mathrm{Boc}}$**H**$_3$), 0.90−0.82 (overlapping t, 6H, CH$_2$C**H**$_3$). ^{13}C NMR ($CDCl_3$): 171.4 (**C**$_{\mathrm{Met}}$═O), 169.1 (**C**$_{\mathrm{Phe}}$═O), 168.8 (**C**$_{\mathrm{Fc}}$═O), 155.3 (**C**$_{\mathrm{Boc}}$═O), 138.2 (**C**$_{\mathrm{Aniline}}$), 136.3 (**C**$_{\mathrm{Phe}}$), 132.5 (**C**$_{\mathrm{Aniline}}$), 129.3, 128.6, 126.9 (**C**$_{\mathrm{Phe}}$), 119.3, 118.0 (**C**$_{\mathrm{Aniline}}$), 89.5 (**C**≡C−CEt$_2$), 83.5 (C≡**C**-CEt$_2$), 80.1 (**C**$_{\mathrm{Boc}}$(CH$_3$)$_3$), 75.9, 70.9, 69.8, 68.3 (**C**$_{\mathrm{Cp}}$), 57.9 (C≡C-**C**Et$_2$), 54.7 (**C**$_{\alpha,\mathrm{Phe}}$), 53.8 (**C**$_{\alpha,\mathrm{Met}}$), 38.1 (**C**$_{\beta,\mathrm{Phe}}$), 31.5 (**C**$_{\beta,\mathrm{Met}}$), 30.6 and 30.5 (**C**H$_2$CH$_3$), 30.1 (**C**$_{\gamma,\mathrm{Met}}$), 28.2 (C$_{\mathrm{Boc}}$(**C**H$_3$)$_3$), 15.2 (S-**C**H$_3$), 8.7 (CH$_2$**C**H$_3$). ^{15}N NMR (in $CDCl_3$): −292 (**N**$_{\mathrm{Boc}}$), −262 (**N**$_{\mathrm{Phe}}$), −255 (**N**$_{\mathrm{DEPA}}$), −253 (**N**$_{\mathrm{Aniline}}$). IR (KBr): 3413 (m), 3324 (m), 1697 (sh), 1650 (s). IR (CH_2Cl_2): 3426 (m), 3370 (sh), 1723 (m), 1675 (br,s). Raman 2228. MS: 815 (M + Na), 792 (M + H). Anal. Calcd for $C_{43}H_{52}N_4O_5FeS$ (792.82 g/mol): C, 65.1; H, 6.6; N, 7.0; Found C, 64.5; H, 7.1; N, 7.3.

7. The dipeptide Boc-Glu(*O*-Bz)-Leu-OMe (4.72 g, 10.16 mmol) was dissolved in 100 mL of methanol in a Schlenk flask. After addition of 1.0 g of Pd/C (10%), the flask was purged with H_2 and the reaction mixture was stirred under H_2 for 15 h. After filtration the filtrate was evaporated to dryness, the oily residue was redissolved in water, and the aqueous solution was slowly acidified by addition of 2 mol/L HCl. The aqueous phase was extracted three times with ether, and the combined organic phases were washed with water, dried over $MgSO_4$, and evaporated to dryness. The resulting product was a clear, colorless oil which was dried for several hours on a vacuum line to yield 2.00 g (5.34 mmol, 53%) Boc-Glu(OH)-Leu-OMe and was used without further purification after establishing its purity by ^{1}H NMR spectroscopy. Boc-Glu(OH)-Leu-OMe was dissolved in 50 mL of thf. After addition of *N*-methylmorpholine (0.54 g, 5.34 mmol), isobutylchloroformate (0.73 g, 5.34 mmol) and 1,1-diethylpropargylamine (0.59 g, 5.34 mmol) stirring was continued for 2 h. Workup as for **5** yielded 0.52 g (1.11 mmol, 21%) of Boc-Glu(NH-CEt$_2$-C≡CH)-Leu-OMe **7** as a white powder. ^{1}H NMR ($CDCl_3$): 6.97 (d, 1H, N$_{\mathrm{Leu}}$**H**, J = 6.8 Hz), 6.01 (br, 1H, N$_{\mathrm{DEPA}}$H), 5.50 (br, 1H, N$_{\mathrm{Boc}}$**H**), 4.51 (mult., 1H, C$_{\alpha,\mathrm{Leu}}$**H**), 4.11 (mult., 1H, C$_{\alpha,\mathrm{Glu}}$**H**), 3.68 (s, 3H, COOC**H**$_3$), 2.33 (s, 1H, C≡C**H**), 2.30 (mult., 2H, C$_{\gamma,\mathrm{Glu}}$**H**$_2$), 2.05 (mult., 3H, C$_{\beta,\mathrm{Glu}}$**H** and C**H**$_2$CH$_3$), 1.95 (mult., 1H, C$_{\beta,\mathrm{Glu}}$**H**), 1.78 (mult., 2H, C**H**$_2$CH$_3$), 1.61 (mult., 3H, C$_{\beta,\mathrm{Leu}}$**H**$_2$ and C$_{\gamma,\mathrm{Leu}}$**H**), 1.39 (s, 9H, C$_{\mathrm{Boc}}$**H**$_3$), 0.94 (mult., 6H, CH$_2$C**H**$_3$), 0.87 (mult., 6H, C$_{\delta,\mathrm{Leu}}$**H**$_2$). ^{13}C NMR ($CDCl_3$): 173.3 (**C**$_{\mathrm{Leu}}$═O), 172.2 (**C**$_{\mathrm{Glu}}$═O), 171.7 (CH$_2$−**C**$_{\mathrm{Glu}}$═O), 155.5 (**C**$_{\mathrm{Boc}}$═O), 85.2 (**C**≡CH), 79.9 (**C**$_{\mathrm{Boc}}$(CH$_3$)$_3$), 71.5 (C≡**C**H), 57.0 (**C**Et$_2$), 53.5 (**C**$_{\alpha,\mathrm{Leu}}$), 52.3 (COO**C**H$_3$), 50.8 (**C**$_{\alpha,\mathrm{Glu}}$), 40.8 (**C**$_{\beta,\mathrm{Leu}}$), 33.0 (**C**$_{\beta,\mathrm{Glu}}$), 30.3 and 30.2 (**C**H$_2$CH$_3$), 28.8 (**C**$_{\gamma,\mathrm{Glu}}$), 28.2 (C$_{\mathrm{Boc}}$(**C**H$_3$)$_3$), 24.7 (**C**$_{\gamma,\mathrm{Leu}}$), 21.6 (**C**$_{\delta,\mathrm{Leu}}$), 8.57 and 8.42 (CH$_2$**C**H$_3$). IR: 3312 (br, m), 1752 (m), 1686 (m), 1654 (s). MS: 467 (8), 411 (9), 295 (67), 239 (33), 195 (56), 57 (100). Anal. Calcd for $C_{24}H_{41}N_3O_6$ (467.61 g/mol): C, 61.7; H, 8.8; N, 9.0. Found: C, 61.6; H, 8.5; N, 8.4.

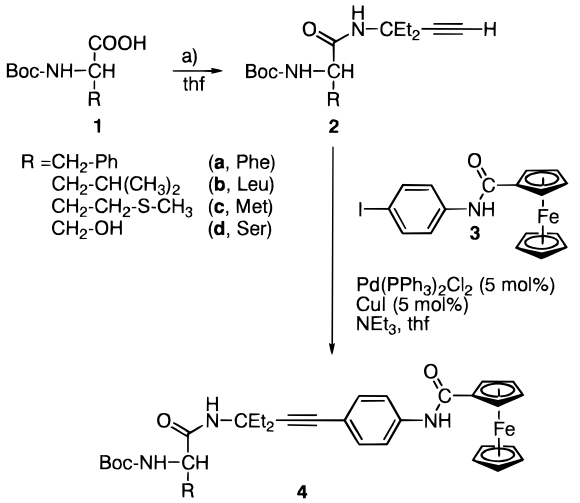
8. Boc-Glu(NH-CEt$_2$-C≡CH)-Leu-OMe **7** (0.52 g, 1.11 mmol) was dissolved in 30 mL of CH_2Cl_2, 10 mL trifluoro acetic acid (TFA) was slowly added at 0 °C, and stirring continued for 30 min. After complete removal of all volatiles on a rotary evaporator, ether was added, stirring of the slurry was continued for 20 min, the solid was filtered off and dried on a vacuum line to yield 0.46 g (0.95 mmol, 87%) of a pure (^{1}H NMR), white triflate salt of H_2N-Glu(NH-CEt$_2$-C≡CH)-Leu-OMe. Boc-Phenylalanine (0.29 g, 1.09 mmol) was dissolved in 50 mL of thf. After addition of 0.11 g (1.09 mmol) of *N*-methylmorpholine and 0.15 g (1.09 mmol) isobutylchloroformate a solution of 1.09 mmol of H_2N-Glu(NH-CEt$_2$-C≡CH)-Leu-OMe in thf (previously neutralized from the triflate salt by addition of NEt_3) was added, and the reaction mixture stirred at room temperature for 60 min. Workup as for **5** gave 0.36 g (0.58 mmol, 61%) of **8** as a white powder. ^{1}H NMR ($CDCl_3$): 7.28−7.19 (mult., 6H, **H**$_{\mathrm{Phe}}$ and N$_{\mathrm{Leu}}$**H**), 7.15 (d, 1H, N$_{\mathrm{Glu}}$**H**, J = 7 Hz), 5.88 (br, 1H, N$_{\mathrm{DEPA}}$**H**), 4.95 (d, 1H, N$_{\mathrm{Boc}}$**H**, J = 7.6 Hz), 4.47 (mult., 1H, C$_{\alpha,\mathrm{Leu}}$**H**), 4.36 (mult., 2H, C$_{\alpha,\mathrm{Glu}}$**H** and C$_{\alpha,\mathrm{Phe}}$**H**), 3.70 (s, 3H, COOC**H**$_3$), 3.06 (mult., 2H, C$_{\beta,\mathrm{Phe}}$H), 2.35 (mult., 2H, C$_{\gamma,\mathrm{Glu}}$**H**$_2$), 2.33 (s, 1H, C≡C**H**), 2.12 (mult., 2H, C**H**$_2$CH$_3$), 2.02 and 1.93 (mult., 1H each, C$_{\beta,\mathrm{Glu}}$**H**), 1.81 (mult., 2H, C**H**$_2$CH$_3$), 1.61 (mult., 3H, C$_{\beta,\mathrm{Leu}}$**H**$_2$ and C$_{\gamma,\mathrm{Leu}}$**H**), 1.37 (s, 9H, C$_{\mathrm{Boc}}$**H**$_3$), 0.97 (mult., 6H, CH$_2$C**H**$_3$), 0.92 (mult., 6H, C$_{\delta,\mathrm{Leu}}$**H**$_2$). ^{13}C NMR ($CDCl_3$): 173.3 (**C**$_{\mathrm{Leu}}$═O), 172.0 (**C**$_{\mathrm{Glu}}$═O), 171.4 (CH$_2$−**C**$_{\mathrm{Glu}}$═O), 170.9 (**C**$_{\mathrm{Phe}}$═O), 155.2 (**C**$_{\mathrm{Boc}}$═O), 136.5, 129.3, 128.7, 127.0 (**C**$_{\mathrm{Phe}}$), 85.1 (**C**≡CH), 80.2 (**C**$_{\mathrm{Boc}}$(CH$_3$)$_3$), 71.7 (C≡**C**H), 57.3 (**C**Et$_2$), 56.0 (**C**$_{\alpha,\mathrm{Leu}}$), 52.3 (COO**C**H$_3$ and **C**$_{\alpha,\mathrm{Glu}}$), 51.1 (**C**$_{\alpha,\mathrm{Phe}}$), 40.7 (**C**$_{\beta,\mathrm{Leu}}$), 38.0 (**C**$_{\beta,\mathrm{Phe}}$), 33.0 (**C**$_{\beta,\mathrm{Glu}}$), 30.4 and 30.3 (**C**H$_2$CH$_3$), 29.0 (**C**$_{\gamma,\mathrm{Glu}}$), 28.2 (C$_{\mathrm{Boc}}$(**C**H$_3$)$_3$), 24.8 (**C**$_{\gamma,\mathrm{Leu}}$), 22.8 and 21.7 (**C**$_{\delta,\mathrm{Leu}}$), 8.63 and 8.60 (CH$_2$**C**H$_3$). IR: 3449 (br, m), 3321 (sh), 1745 (w), 1736 (sh), 1686 (m), 1647 (s). Raman: 2115. MS: 614 (25), 523 (17), 442 (19), 394 (100), 195 (32), 153 (43), 120 (59), 57 (91). Anal. Calcd for $C_{33}H_{50}N_4O_7$ (614.78 g/mol): C, 64.5; H, 8.2; N, 9.1. Found: C, 64.6; H, 8.3; N, 9.1.

9.. The synthesis was carried out analogous to **6**, using 126 mg (0.29 mmol) of **3**, 5 mg (0.03 mmol) CuI, 10 mg (0.015 mmol) $(Ph_3P)_2PdCl_2$, and 180 mg (0.29 mmol) of Boc-Phe-Glu(NH-CH$_2$-C≡CH)-Leu-OCH$_3$ (**8**). Analytical HPLC and ^{1}H NMR both showed the product to be >90% pure **9**, and only a small amount from the HPLC (conditions as for **6**) was used to obtain analytical data. ^{1}H NMR ($CDCl_3$): 7.54 (pseudo-d, 2H, **H**$_{\mathrm{Aniline}}$), 7.40 (pseudo-d, 2H, **H**$_{\mathrm{Aniline}}$), 7.37 (s, 1H, N$_{\mathrm{Aniline}}$**H**), 7.34−7.14 (mult., 7H, **H**$_{\mathrm{Phe}}$, N$_{\mathrm{Leu}}$**H**, N$_{\mathrm{Glu}}$**H**), 5.94 (br, 1H, N$_{\mathrm{DEPA}}$**H**), 5.27 (br, 1H, N$_{\mathrm{Boc}}$**H**), 4.75 (pseudo-t, 2H, **H**$_{\mathrm{Cp}}$), 4.47−4.34 (mult., 3H, C$_{\alpha,\mathrm{Leu}}$**H,** C$_{\alpha,\mathrm{Phe}}$**H**, C$_{\alpha,\mathrm{Glu}}$**H**), 4.42 (pseudo-t, 2H, **H**$_{\mathrm{Cp}}$), 4.24 (s, 5H, **H**$_{\mathrm{Cp}}$), 3.69 (s, 3H, COOC**H**$_3$), 3.05 (mult., 2H, C$_{\beta,\mathrm{Phe}}$**H**$_2$), 2.38−2.20 (mult., 4H, C$_{\gamma,\mathrm{Glu}}$**H**$_2$ and C**H**$_2$−CH$_3$), 1.98 (mult, 2H, C$_{\beta,\mathrm{Glu}}$**H**), 1.88 (mult., 2H, C**H**$_2$−C**H**$_3$), 1.60 (mult, 3H, C$_{\beta,\mathrm{Leu}}$**H**$_2$ and C$_{\gamma,\mathrm{Leu}}$**H**), 1.37 (s, 9H, C$_{\mathrm{Boc}}$**H**$_3$), 1.02 (mult., 6H, CH$_2$C**H**$_3$), 0.92 (overlapping t, 6H, C$_{\delta,\mathrm{Leu}}$**H**). ^{15}N NMR ($CDCl_3$): −293 (**N**$_{\mathrm{Boc}}$), −262 (**N**$_{\mathrm{Glu,}}$ **N**$_{\mathrm{Leu}}$), −254 (**N**$_{\mathrm{Aniline}}$), −254 (**N**$_{\mathrm{DEPA}}$). Raman 2230. MS: 918 (M + H)$^+$, 940 (M + Na)$^+$. High-resolution. MS calcd. for $C_{50}H_{63}N_5O_8Fe$ (917.92 g/mol): 940.3924 for (M + Na)$^+$. Found 940.3935.

Results and Discussion

We have chosen the C-terminus of amino acids as the site of labeling, thus a number of different enantiomerically pure Boc-protected amino acids **1** were reacted with 1,1-diethylpropargylamine using the isobutylchloroformate/*N*-methylmorpholine protocol (Boc = *tert*-butoxycarbonyl) (Scheme 1). The *N*-Boc-protected alkynyl amino acids **2** were obtained almost quantitatively as pure, white crystalline materials after recrystallization from hot thf/heptane. The coupling with (*p*-iodoanilido)ferrocene carboxylic acid **3** was carried out in hot thf in the presence of 5 mol % of $PdCl_2(PPh_3)_2$/CuI. After workup, ferrocene amino acids **4** were isolated as orange powders. It should be noted that this reaction does not require dry, purified solvents (the reactions were normally carried out in reagent grade thf without

Scheme 1. Formation of Alkynyl Amino Acids **2** and Alkynyl Amino Acid Ferrocene Conjugates **4**



a) ^{i}BuO-C(O)-Cl, N-Methyl-morpholine,H_2N-CEt$_2$-C≡C-H

further purification except for deaeration). Furthermore, there is a wide tolerance of functional groups for the Pd catalyst which tolerates alcohols (R = -CH_2OH, Ser, **4d**) and thioethers (R = -CH_2-CH_2-S-CH_3, Met, **4c**) and, of course, amides in the coupling reaction (Scheme 1).

Mass spectrometry (MS) is a valuable tool for confirming that a coupling according to Scheme 1 has indeed taken place. The results of two ionization methods, namely electron ionization (EI) and electrospray ionization (ESI), were helpful for establishing the constitution of compounds **4**. In the ESI-MS spectra, the intact compounds were detected almost exclusively as their H^+, Na^+, or K^+ adduct ions. In contrast, EI-MS spectra showed characteristic fragment ions. By comparison with reference compounds such as alkynyl amino acids **2**, bonding of the ferrocene carboxylic acid anilide to the alkyne was confirmed by the presence of related fragment ions. Further proof comes from NMR spectroscopy, namely, the ^{1}H signal of the terminal alkyne. In **2**, this signal is observed at ca. 2.3 ppm in $CDCl_3$, with a notable solvent dependence of the chemical shift value (ca. 3.1 ppm in DMSO solution). This appears to be an exceptionally large shift range and has been attributed to the formation of hydrogen bridges with solvents such as DMSO. Evidently, this signal is lost in conjugates **4**, while most of the other proton NMR signals change little after the coupling. It should be noted, that the ethyl groups in **2** and **4** are diastereotopic due to the presence of the chiral C_α carbon in the amino acids and hence give rise to two groups of signals. Two other significant changes are observed in the ^{13}C NMR spectra of **2** and **4**. Upon going from iodo to alkyne substitution the signal of the *ipso* carbon center of the aromatic ring experiences a downfield shift of about 30 ppm, and the ^{13}C NMR resonance of the terminal alkyne carbon moves ca. +18 ppm to lower field. The resonance of the internal carbon of the alkyne shifts only about 1 ppm to higher field.

We have used 2D indirect detection ^{1}H−^{15}N NMR spectroscopy to determine the chemical shift for all ^{15}N resonances. Typical values of −292 ppm vs nitromethane are found for the Boc-protected nitrogen atom, values around −262 ppm for amide bonds of the peptide backbone, −255 ppm for the propargyl amide nitrogen center and −253 ppm for the anilide nitrogen atom.[26,27] Coupling constants $^1J(^1\text{H}-^{15}\text{N})$ are typically found to be 90 Hz with little variation. From these values there

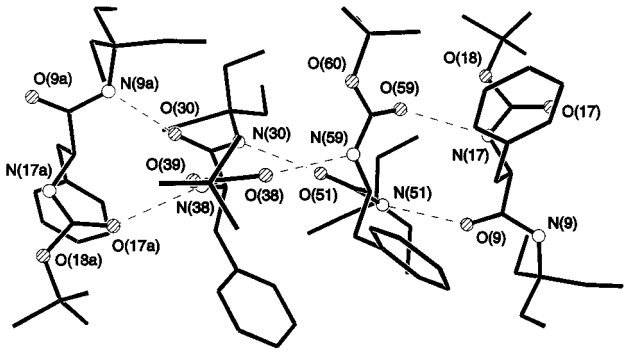
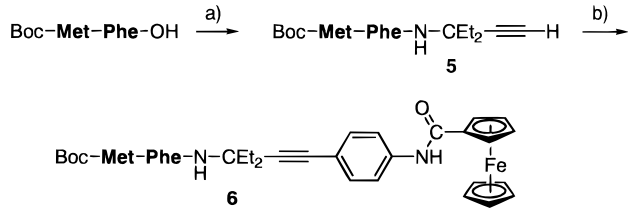


Figure 1. Plot of the solid-state structure of **2a**, showing the helix which forms by hydrogen bonding. Selected bond lengths (Å): N(9a)−O(30), 2.892; O(17a)−N(38), 2.893; N(30)−O(51), 2.904; O(38)−N(59), 2.925; N(51)−O(9), 2.948; O(59)−N(17), 2.867.

Scheme 2. Synthesis of Dipeptide **5** and the Ferrocene-Labeled Dipeptide **6**

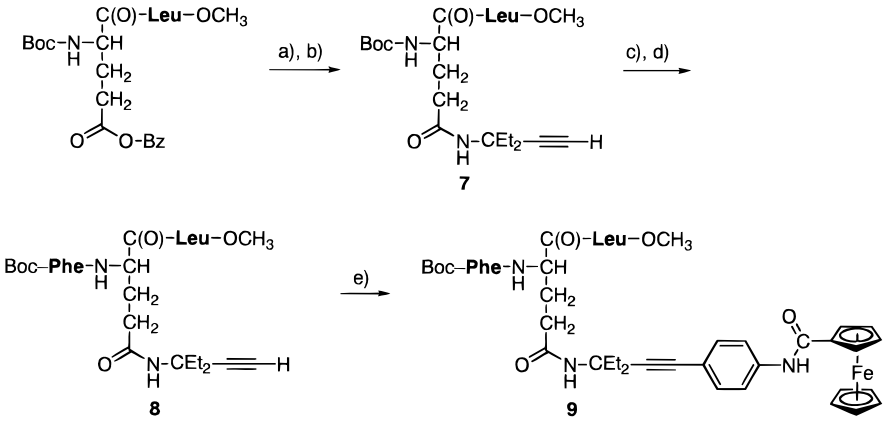


a) ^{i}BuO-C(O)-Cl, N-Methyl-morpholine,H_2N-CEt$_2$-C≡C-H
b) $(PPh_3)_2PdCl_2$ / CuI (5 mol%), NEt_3, thf, **3.**

is no indication that intra- or intermolecular hydrogen bonding plays a significant role for compounds **4** in solution. This view is substantiated by infrared (IR) spectra in CH_2Cl_2 solution. For alkynyl amino acids **2** as well as for ferrocene derivatives **4** we find N−H stretching vibrations with wavenumbers >3400 cm^{-1}. The absence of any bands below 3400 cm^{-1} is an indication for the lack of hydrogen bridges in solution.[8,28,29] However, solid-state IR spectra of **2** and **4** clearly show medium intensity bands below 3400 cm^{-1} which indicate that the amide hydrogen atoms are involved in hydrogen bonds in the solid state. We have carried out an X-ray single-crystal structure determination on **2a** to determine the nature of these hydrogen bonds. While the geometry of **2a** does not show any unusual features, **2a** forms hydrogen bonds such that the three crystallographically independent molecules form a left-handed helical structure along the crystallographic screw axis parallel to the *c* axis (Figure 1). Accordingly, six amino acid molecules complete one helical turn in 26.65 Å, which corresponds to the length of the crystallographic *c* axis. Despite various efforts, we were unable to grow crystals of suitable quality for an X-ray single-crystal structure determination of **4**, but based on the crystal structure of **2a** and our IR experiments we expect a similar hydrogen bonding network for compounds **4** in the solid state. The potential of such supramolecular assemblies derived from amino acids, for example on solid surfaces, has recently been pointed out by Schade and Fuhrhop.[30]

(26) Witanowski, M.; Stefaniak, L. *Annu. Rep. NMR Spectrosc.* **1986**, *18*, 1−761.
(27) Witanowski, M.; Stefaniak, L.; Webb, G. A. *Annu. Rep. NMR Spectrosc.* **1993**, *25*, 1−480.
(28) Liang, G.-B.; Dado, G. P.; Gellman, S. H. *J. Am. Chem. Soc.* **1991**, *113*, 3994−3995.
(29) Gellman, S. H.; Dado, G. P.; Liang, G.-B.; Adams, B. R. *J. Am. Chem. Soc.* **1991**, *113*, 1164−1173.
(30) Schade, B.; Fuhrhop, J.-H. *New J. Chem.* **1998**, 97−104.

Scheme 3. Synthesis of Tripeptide **8** and Its Labeling with Ferrocene to Yield the Ferrocene-Labeled Tripeptide **9**



a) H_2, Pd / C (10 %), thf; b) ^{i}BuO-C(O)-Cl, N-Methyl-morpholine,H_2N-CEt_2-C≡C-H
c) CF_3CO_2H, CH_2Cl_2 (1 : 1); d) ^{i}BuO-C(O)-Cl, N-Methyl-morpholine,Boc-**Phe**-OH
e) $(PPh_3)_2PdCl_2$ / CuI (5 mol%), NEt_3, thf, **3.**

We have recorded a ^{57}Fe Mössbauer spectrum for **4a**. The isomer shift (0.52 mm s^{-1}) and quadrupole splitting (2.32 mm s^{-1}) are very similar to the values for ferrocene (0.53 and 2.37 mm s^{-1}),[31] suggesting that there is little influence of the overall solid-state structure of **4a** on the field gradients of the iron nucleus. As we are interested in electrochemical detection of ferrocene-labeled biomolecules,[32] we have determined the potential for one-electron oxidation of the ferrocene moiety by cyclic voltammetry (CV). For **4a**−**d**, a reversible one-electron oxidation occurs around +190 mV vs ferrocene/ferrocenium. The carboxylic acid anilide acts as an electron withdrawing substituent to the ferrocene moiety, but its oxidation potential is not significantly influenced by the nature of the adjacent amino acid.[9,33] This is further emphasized by a comparable potential of +189 mV for **3**. A second, irreversible wave in the CV of **4c** is attributed to oxidation of the sulfur atom.

After establishing the necessary reaction conditions on amino acids as model compounds we then used the methodology outlined above for the labeling of a dipeptide and a tripeptide as follows. The *N*-Boc-protected dipeptide Boc-Met-Phe-OH was prepared by standard methods,[34] activated with isobutylchloroformate and reacted with 1,1-diethylpropargylamine to give **5**. Under Pd catalysis, **5** could be coupled to **3** to give the ferrocene-labeled dipeptide **6** (Scheme 2).

Diethyl ether extraction of the product following $H_2O/CHCl_3$ workup yielded analytically pure compounds **4**. This protocol fails in the case of **6** and **9** as these compounds are insoluble in ether. However, **6** and **9** were shown to be >90% pure by ^{1}H NMR spectroscopy and HPLC, and analytically pure samples were obtained from HPLC (see Experimental Section). Four ^{15}N NMR signals are observed and their position is characteristic for the constitution of **6**: the Boc amide signal (−292 ppm vs CH_3NO_2), the internal amide bond (−262 ppm), the propargyl amide which is observed at −255 ppm, and the ferrocene carboxylic acid anilide signal at −253 ppm. To demonstrate that our methodology can also be applied to nonterminal positions of a peptide we have synthesized the tripeptide Boc-Met-Glu(NH-CEt_2-C≡CH)-Leu-OCH_3 **8** as outlined in Scheme 3.

It is noteworthy that the alkyne is already introduced during the course of the synthesis and stable under the subsequent steps, namely Boc-deprotection by TFA in CH_2Cl_2, and coupling to an activated acid. The tripeptide **8** was coupled to ferrocene carboxylic acid *p*-iodoanilide **3** under Pd catalysis and the formation of the ferrocene-labeled tripeptide **9** was established by high-resolution MS. A complete assignment of the ^{1}H NMR signals was achieved by H−H COSY, H−C and H−N correlated 2D spectra. The ^{1}H−^{15}N HSQC spectrum of **9** shows five cross-peaks as expected, corresponding to the Boc amide (δ^{15}N −293), two overlapping resonances for the peptide backbone (δ^{15}N −262), and the propargylamide signal, again at slightly higher field (−253 ppm). Finally, the signal at −254 ppm originating from the ferrocene amide supports the mass spectroscopic result that the bulk of peptide **9** is labeled by ferrocene. In addition, the C≡C stretching frequency shifts from the value for the terminal alkyne (2115 cm^{-1}) in **8** to 2230 cm^{-1} in the coupling product **9** as established by Raman spectroscopy.

In this communication a new principle for the formation of organometallic peptides is introduced as exemplified by the labeling of a variety of amino acids with a ferrocene derivative. However, there is no principal limitation to this concept as shown by the successful labeling of a di- and a tripeptide and we are currently extending this chemistry to other organometallics and biomolecules.

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Supporting Information Available: Complete listings of crystal data and refinement details, atomic coordinates and isotropic displacement parameters, bond lengths and angles and anisotropic displacement parameters. This material is available free of charge via the Internet at http://pubs.acs.org.

IC990256+

(31) Greenwood, N. N.; Gibb, T. C. *Mössbauer Spectroscopy*; Chapman and Hall: London, 1971.
(32) Ihara, T.; Nakayama, M.; Murata, M.; Nakano, K.; Maeda, M. *J. Chem. Soc., Chem. Commun.* **1997**, 1609−1610.
(33) Lin, L.; Berces, A.; Kraatz, H.-B. *J. Organomet. Chem.* **1998**, *556*, 11−20.
(34) Bodanszky, M.; Bodanszky, A. *The Practice of Peptide Synthesis*; Springer-Verlag: Berlin, 1984.