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Click Chemistry Inspired Synthesis of Novel Ferrocenyl-Substituted Amino Acids or Peptides

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This work reports on the synthesis of a wide range of ferrocenyl-substituted amino acids and peptides in excellent yield. Conjugation is established via copper-catalyzed 1,3-dipolar cycloaddition. Two complementary strategies were employed for conjugation, one involving cycloaddition of amino acid derived azides with ethynyl ferrocene **1** and the other involves cycloaddition between amino acid derived alkynes with ferrocene-derived azides **2** and **3**. Labeling of amino acids at multiple sites with ferrocene is discussed. A new route to 1,1′-unsymmetrically substituted ferrocene conjugates is reported. A novel ferrocenophane **19** is accessed via bimolecular condensation of amino acid derived bis-alkyne **9b** with the azide **2**. The electrochemical behavior of some selected ferrocene conjugates has been studied by cyclic voltammetry.

Introduction

The robustness of ferrocene under aerobic conditions, the easy access to many of its derivatives and their favorable electrochemical properties have made ferrocene a favorite molecule for conjugation on to biomolecules like amino acids, peptides, proteins, carbohydrates, DNA and RNA.[1] In general, their increased biological activity in comparison with the unconjugated biomolecules has been well documented in the literature.[1] Labeling biomolecules with ferrocene is helpful for their electrochemical detection,[2] for immunoassays[3] or for spectroscopic techniques like IR[4] and optical spectroscopy.[5] A number of ferrocene-derived peptides were used as electrochemical probes.[6] Many biologically important oligopeptides like [Leu^5]-enkephalin,[7] bradykinin,[8] angiostatin II[9] were labeled with ferrocene either by replacing the parent amino acid of the peptide with ferrocene-derived amino acids or by covalent attachment of ferrocene derivatives onto the peptides via known methodologies and their activity was studied. In his pioneering review,[1a] Metzler-Nolte has compiled various methods available in the literature for covalently binding ferrocene on to biomolecules and their applications. Metzler-Nolte et al. have documented in three different reports[10a–10c] the palladium-catalysed Sonogashira coupling scheme for the synthesis of ferrocene-labeled amino acids and peptides. Some of their coupling products were inseparable from the impurities and hence, analytically pure samples could not be obtained. Additionally, their method was limited to mono-substituted ferrocene derivatives and side chains of various amino acids were not used for labeling. While this manuscript was under preparation, Metzler-Nolte et al. reported the spectroscopic and electrochemical studies of ferrocene–amino acid conjugates using click chemistry. They used azidoferrocene and 1,1′-diazidoferrocene for labeling.[10d] Reported methods for labeling amino acids and peptides are specific to either *N*[1a,11] or *C*[12] terminus or side chains.[13] To explore the area of bioorganometallic chemistry further, new ways which are more general are necessary. Copper(I)-catalysed Huisgen reaction between a terminal alkyne and an azide which furnishes a 1,4-substituted triazole derivative has proven to be a powerful tool for the synthesis of conjugates.[14] Herein, we report on the selective functionalization of amino acids and peptides with ferrocene using 1-ethynylferrocene (**1**) and the azides **2**, **3** (see Scheme 1) as ferrocene labels. The electrochemical behavior of a few representative ferrocene conjugates was studied by using cyclic voltammetry. Towards this goal two complementary strategies were designed. The first one in-

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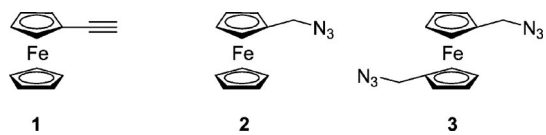
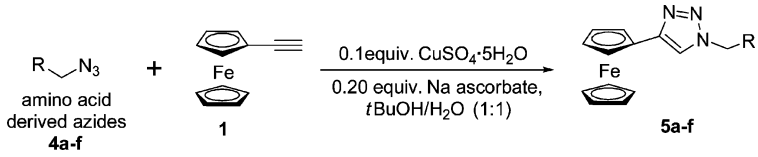
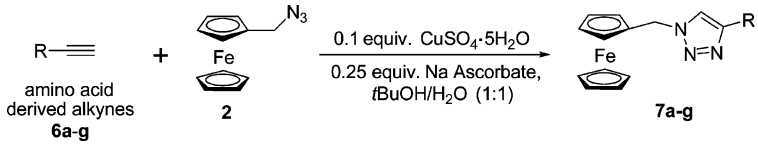


Scheme 1. Ferrocene derivatives employed for labeling.



Scheme 2. General scheme for the synthesis of ferrocene conjugates **5a**–**f** derived from **1**.



Scheme 3. General scheme for the synthesis of ferrocene conjugates **7a**–**g** derived from **2**.

volves labeling of the amino acid/peptide derived azides at various positions with ethynyl ferrocene **1** (Scheme 2). The second strategy involves labeling the amino acid/peptide derived terminal alkynes with azides **2** and **3** to furnish monovalent and divalent ferrocene conjugates, respectively (Scheme 3).

Results and Discussion

Synthesis of Ferrocene Conjugates from Ethynylferrocene (1)

Initially, azides present on *N*-terminus of various amino acids **4a**–**e** and peptide **4f** were synthesized. For this, methyl esters of L-phenylalanine, valine and aspartic acid were treated with chloroacetyl chloride to furnish the corresponding chloroacetamides which were treated with NaN_3 in DMF to furnish the corresponding azidoacetamides **4a**–**c**.[15a]

When **4a** was treated with **1** (1 equiv.) in *t*BuOH/H_2O (1:1) with $CuSO_4{\cdot}5H_2O$ (0.1 equiv.) as catalyst and sodium ascorbate (0.25 equiv.) as oxidant and was allowed to stir at room temperature for 4 h furnished the corresponding conjugate **5a** in 95% yield (Table 1). Similarly, **5b** and **5c** were synthesized in high yield starting from **4b** and **4c** respectively. *N*-Cbz-protected L-alanine and phenylglycine were coupled with 2-bromoethanol using DCC to synthesize bromoethyl esters[15b] of the amino acids which were treated with NaN_3 in DMF to furnish *C*-terminus azides **4d**,**e** which were then treated with **1** in a similar fashion to furnish alanine and phenyl glycine-conjugated to ferrocene **5d**,**e** via *C*-terminus as the only products (Table 1). The azide-containing dipeptide (N_3-CH_2-CO-Phe-Val-OMe) **4f** was also synthesized and conjugated to **1** to furnish **5f** in excellent yield (93%). The *ipso*-C on the Cp ring for the conjugates **5a**–**f** derived from **1** resonated around δ = 75 ppm in ^{13}C NMR spectrum and the hydrogen atom on triazole ring resonated around δ = 7.5–7.7 ppm in the ^1H NMR spectrum.

Synthesis of Ferrocene Conjugates Derived from 2 and 3

After the successful synthesis of ferrocene conjugates using **1**, the utility of compounds **2** and **3** was explored. For this suitably protected amino acid derived alkynes **6a**–**g** were synthesized.

Propargyl carbamates from L-methionine and tryptophan[15c] **6a** and **b**, *N*-propargyl leucine[15d] **6c**, dipeptide (Poc-phe-val-OMe)[15c] **6g** were synthesized for *N*-terminus conjugation with **2** and **3** (Figure 1). The propargyl ester of *N*-benzoyl-L-phenylalanine[15e] **6d** was synthesized for conjugation via *C*-terminus. The side chains of serine and tyrosine were protected as propargyl carbonates **6e**,**f** and were made viable for conjugation via side chain of amino acids. This is the first report of conjugation involving side chains

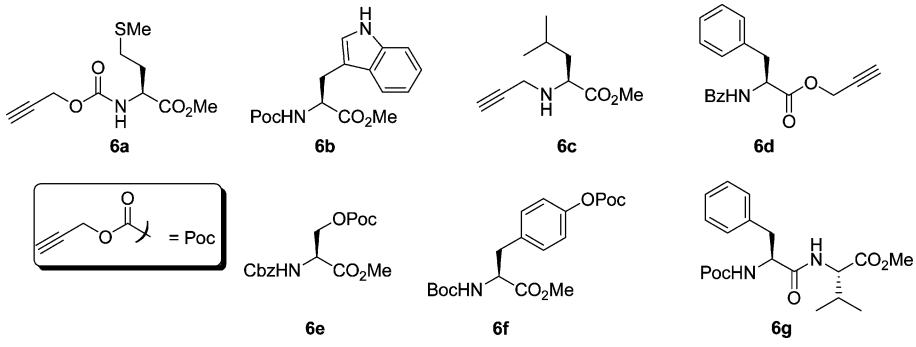
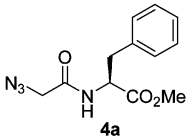
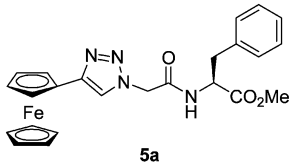
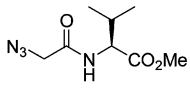
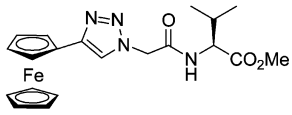
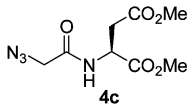
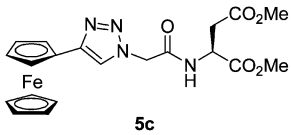
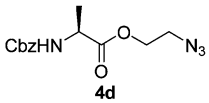
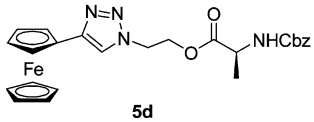
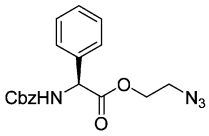
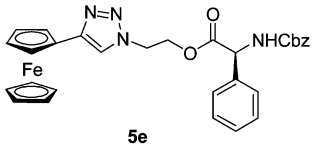
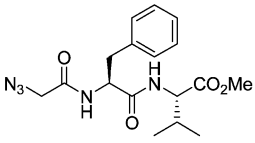
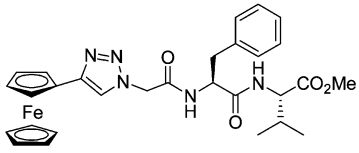


Figure 1. Alkynes **6a**–**g** derived from amino acids.

Table 1. Conjugates **5a**–**f** of amino acid derived azides with **1**.

Substrate	Product	Time / h	% Yield
 **4a**	 **5a**	4	95
 **4b**	 **5b**	4	96
 **4c**	 **5c**	4	93
 **4d**	 **5d**	6	92
 **4e**	 **5e**	6	95
 **4f**	 **5f**	6	93

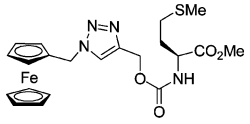
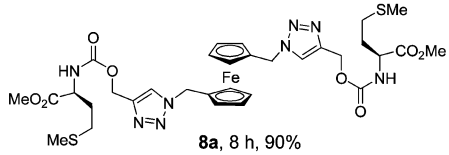
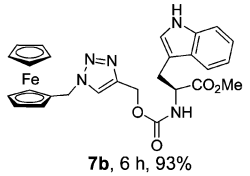
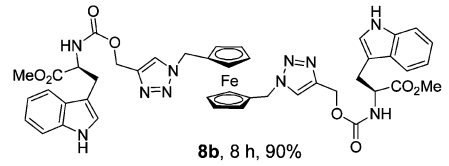
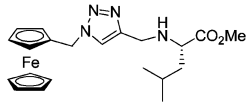
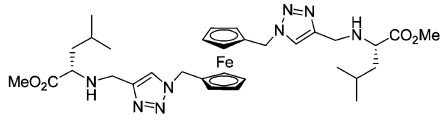
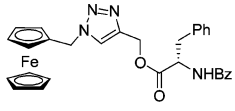
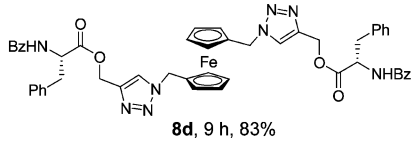
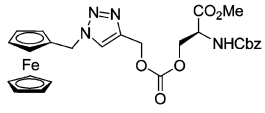
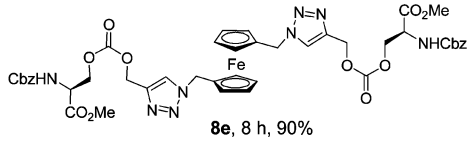
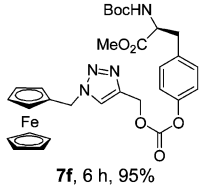
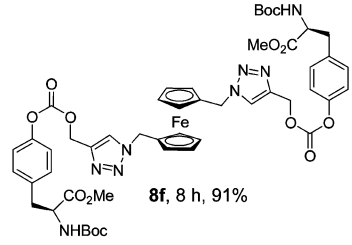
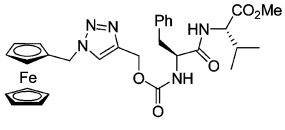
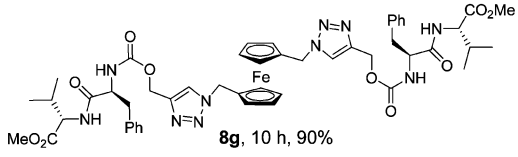
of serine and tyrosine. In this strategy, alkynes of different types like carbamates **6a**,**b**,**g**, carbonates **6e** and **f**, alkylamines **6c**, ester **6d** were used. Initially methionine derived alkyne **6a** was treated with 1 equiv. of azide **1** (Table 2) to furnish compound **7a** in 92% yield.

In a similar fashion, treating alkynes **6b**–**f** with **2** furnished conjugates **7b**–**f** in excellent yield. For the synthesis of disubstituted conjugates, **6a** (1 equiv.) was treated with azide **3** (0.5 equiv.) in *t*BuOH/H_2O (1:1) and was allowed to stir at room temperature for 8 h to furnish the conjugate **8a** (90%) which has methionine substituted on both rings of ferrocene. (Table 2). In a similar fashion, **6b**,**c** and **g** on reaction with **2** and **3** furnished tryptophan and leucine and dipeptide-conjugated ferrocenes **7b**,**c** and **g**, **8b**,**c** and **g** via their *N*-terminus in excellent yield (Table 2). The propargyl ester **6d** was subjected to the click reaction with **2** and **3** to give phenylalanine-conjugated ferrocenes **7d**, **8d** via *C*-terminus. For conjugates synthesized from side chains of amino acids, serine- and tyrosine-derived carbonates **6e**,**f** were subjected to click reaction with **2** and **3** to furnish conjugates **7e**,**8e** and **7f**,**8f** (Table 2). The *ipso*-C of the Cp ring resonated around δ = 80–82 ppm in ^{13}C NMR spectrum whereas the triazole hydrogen resonated around δ = 7.4–7.6 ppm in ^{1}H NMR spectrum which is characteristic of the conjugates derived from **2** and **3**.[14f]

Amino Acid Conjugates Containing the Ferrocene Label at More Than One Site

After the successful synthesis of mono and disubstitued conjugates of ferrocene **7a**–**g**, **8a**–**g** with amino acids and peptides via their *N*/*C* terminus and side chains, our next target was to label amino acids with ferrocene at more than one site. For this purpose, compounds **9a**–**d** were synthesized. Compound **9a** which has two azide moieties incorporated was synthesized from L-lysine, while **9b** containing one propargyl carbamate and propargyl ester was derived from L-phenylalanine. Reaction of tyrosine methyl ester with Poc-Cl (propargyloxycarbonyl chloride) furnished **9c** where the *N*-terminus is protected as carbamate and the side-chain phenol is protected as carbonate. Similarly, the

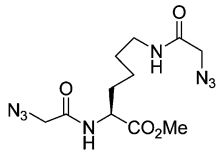
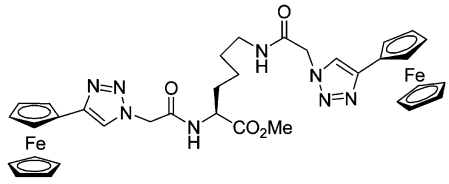
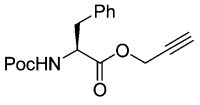
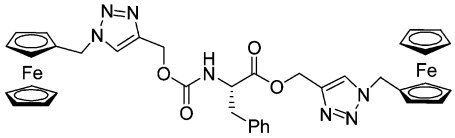
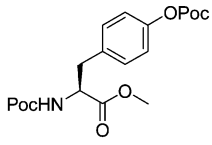
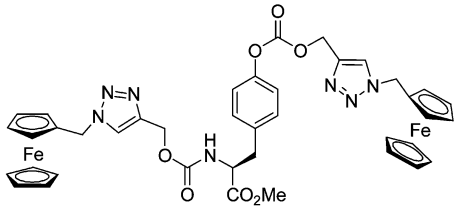
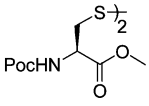
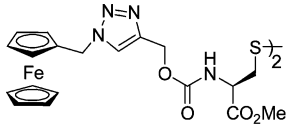
Table 2. Synthesis of monosubstituted **7a**–**g** and disubstituted conjugates **8a**–**g** from **2** and **3**.

Substrate	Monovalent conjugates	Divalent conjugates
6a	 **7a**, 6 h, 92%	 **8a**, 8 h, 90%
6b	 **7b**, 6 h, 93%	 **8b**, 8 h, 90%
6c	 **7c**, 8 h, 88%	 **8c**, 10 h, 84%
6d	 **7d**, 7 h, 86%	 **8d**, 9 h, 83%
6e	 **7e**, 6 h, 94%	 **8e**, 8 h, 90%
6f	 **7f**, 6 h, 95%	 **8f**, 8 h, 91%
6g	 **7g**, 8 h, 92%	 **8g**, 10 h, 90%

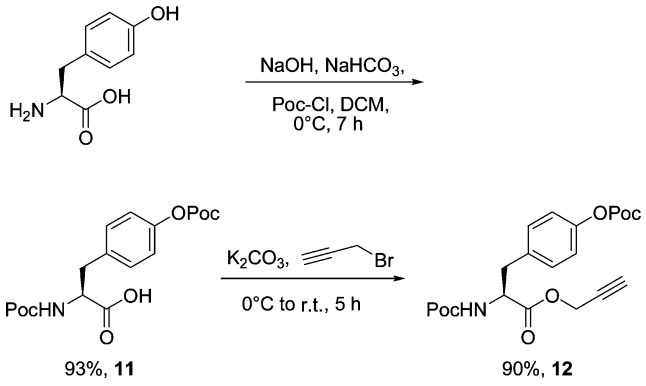
di-Poc derivative **9d** was synthesized from L-cystine. Treatment of **9a** with alkyne **1** (2.1 equiv.) under the same catalytic conditions in *t*BuOH/H_2O (1:1) gave the bimetallic conjugate of lysine **10a** in 90% yield (Table 3). Bis-alkynes derived from phenylalanine, tyrosine and cystine, **9b**–**d** were treated with azide **2** (2.1 equiv.) to furnish conjugates **10b**–**d** in excellent yield (Table 3).

While Meltzler-Nolte in his work could not isolate a doubly labeled ferrocene conjugate of cystine by Sonogashira coupling,[10b] we could easily synthesize **10d** in 86% yield in high purity under the click chemistry inspired synthesis demonstrating the advantage of our methodology. Then our attention was directed towards the synthesis of trimetallic conjugates. For this purpose amino acid derivatives containing three terminal alkynes were synthesized. Tyrosine was converted into its di-Poc derivative **11** (*N*-Poc and *O*-Poc) using Poc-Cl which was treated with propargyl bromide to furnish compound **12** (Scheme 4) in 90% yield. Similarly, compound **13** was prepared in two steps from lysine. When compound **12** was treated with **2**

Table 3. Synthesis of divalent conjugates from amino acid derived bis-azides **9a** and bis-alkynes **9b–d**.

Substrate	Divalent conjugates	Time / h	% Yield
 **9a**	 **10a**	7	90
 **9b**	 **10b**	8	88
 **9c**	 **10c**	8	86
 **9d**	 **10d**	9	86

(3.3 equiv.) under Sharpless conditions novel trimetallic compound **12a** was obtained in 85% yield after 24 h (Scheme 5).



Scheme 4. Synthesis of tris-alkyne **12** derived from tyrosine.

This we believe is the first report of an amino acid which is labeled at multiple sites with ferrocene. The compounds **7f**, **10c** and **12a** form a series of compounds where tyrosine is labeled with one, two and three ferrocene units respectively. Similarly, compound **13** derived from lysine under similar conditions furnished **13a** in 82% yield (Scheme 6).

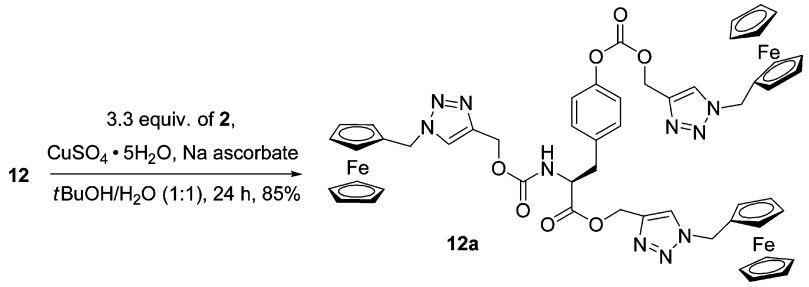
Divergent Syntheses of Dipeptide Ferrocene Conjugate 14c

Previously, in a convergent approach, we have synthesized ferrocene conjugate **7g** from dipeptide **6g** by click reaction with ferrocenyl methyl azide **2**. Alternatively, we wished to test whether the free amino acid can be tagged on to ferrocene and then grow the peptide chain in a divergent fashion. Accordingly, Poc-protected alanine **14** was treated with **2**(1.0 equiv.) with CuI as catalyst and DIPEA as base to generate **14a** followed by addition of valine methyl ester **14b** in to the same pot under peptide coupling conditions (NMM, HOBt, EDC·HCl, 5 h) afforded dipeptide conjugate **14c** in 80% yield (Scheme 7).

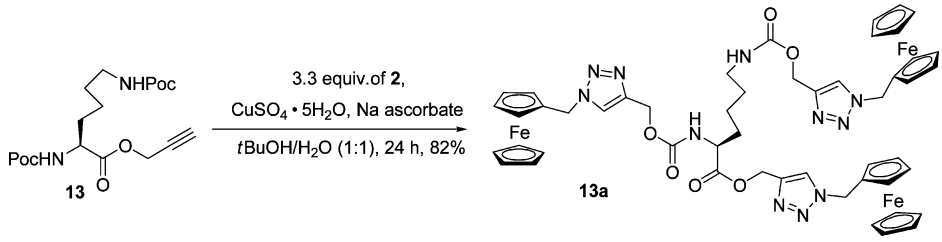
Synthesis of Novel Unsymmetrical Ferrocene Conjugates 17 and 18

There are no reports in the literature on the synthesis of unsymmetrical ferrocene conjugates (with different substituents on each ring). Attempted synthesis of unsymmetrical diglyco-ferrocene conjugates by Casas-Solvas using a different approach failed.[14f] We anticipated that our approach via click chemistry can overcome that problem.

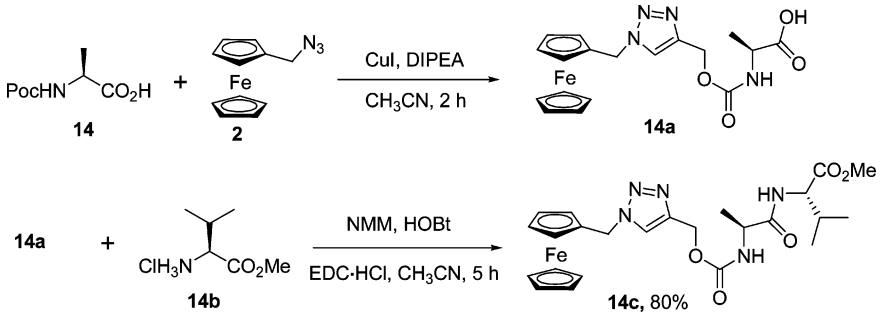
In order to achieve this, Poc-protected phenylalanine methyl ester **16** was treated with azide **3** (5 equiv.) under



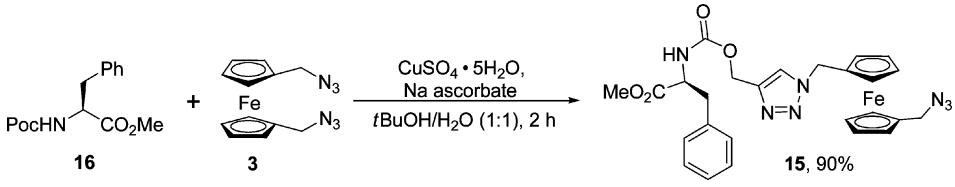
Scheme 5. Synthesis of the tris-labeled conjugate **12a** derived from tyrosine.



Scheme 6. Synthesis of tris-labeled conjugate **13a** derived from L-lysine.



Scheme 7. Synthesis of ferrocene-labeled dipeptide **14c** in a divergent manner.



Scheme 8. Monosubstituted ferrocene conjugate of phenylalanine **15** from bis-azide **3**.

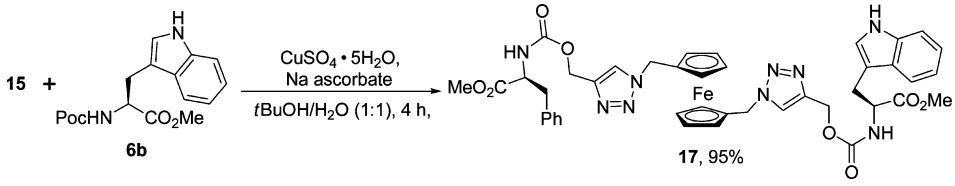
Sharpless conditions to furnish the monosubstituted derivative **15** in excellent yield (Scheme 8). When this compound was subjected to click reaction with Poc-protected tryptophan **6b** (1 equiv.) we could isolate the unsymmetrically substituted conjugate **17** in excellent yield (Scheme 9). In compound **17**, both the amino acids were conjugated via their *N*-termini which can be considered as two parallel strands grown on ferrocene having carboxylic groups at the end.

To synthesize conjugates containing antiparallel strands on ferrocene, compound **15** was treated with *N*-benzoyl propargyl ester **6d** (1 equiv.) under click chemistry conditions to yield compound **18** in excellent yield (Scheme 10).

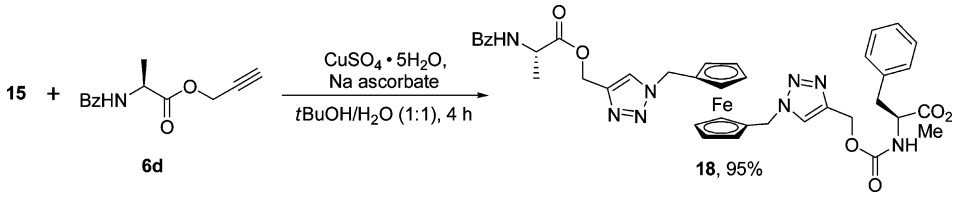
Finally, we tested whether the reaction of azide **3** with bis-alkyne **9b** derived from phenylalanine could furnish a chiral macrocycle. Accordingly, when this reaction was performed under normal dilution only traces of ferrocenophane **19** was obtained, but under high dilution conditions, the novel macrocyclic ferrocenophane **19** was obtained in 20% yield (Scheme 11).

Electrochemical Characterization

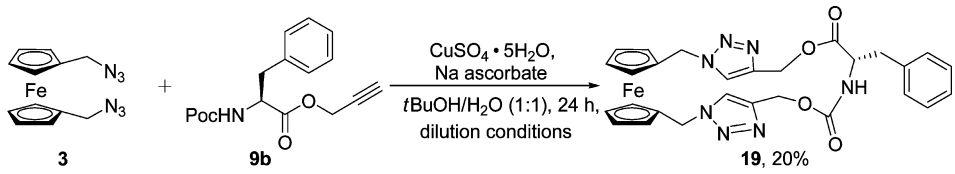
A representative set of molecules was characterized electrochemically and their structure–redox activity relation-



Scheme 9. Synthesis of unsymmetrical parallel-stranded ferrocene conjugate **17** from **15**.



Scheme 10. Synthesis of unsymmetrical, antiparallel-stranded ferrocene conjugate **18**.



Scheme 11. Novel ferrocenophane **19** from bis-azide **3** and bis-alkyne **9b**.

ship was studied. Compounds **7b**, **8b**, **7f**, **10c**, **12a**, **5f** and **7g** were studied using cyclic voltammetry. The voltammograms were recorded for the above-mentioned compounds in CH_3CN (5 mL), with Bu_4NPF_6 as the supporting electrolyte (0.2 M) and an analyte concentration of 1 mM.

The working electrode was a gold disc, the counter electrode was a large-area Pt foil, and the reference electrode was Ag/AgCl/0.1 M Bu_4NPF_6 in acetonitrile. The measurements revealed a one-electron, fully reversible behavior for the ferrocenyl group for all the compounds. The voltammogram of **8b** showed higher redox potential than that of **7b** (Figure 2) due to the presence of substitution on both the rings. It was expected that the CV of compound **10c**, might show two peaks for the two ferrocene moieties while compound **12a** might reveal three peaks due to three ferrocene moieties present. However, the voltammograms (Figure 3) revealed only increase in currents going from **7f** to **12a** corresponding to the increased redox concentration but did not show different peaks. We attribute this to the similar electronic environment of ferrocene moieties in the molecules though they are chemically different.

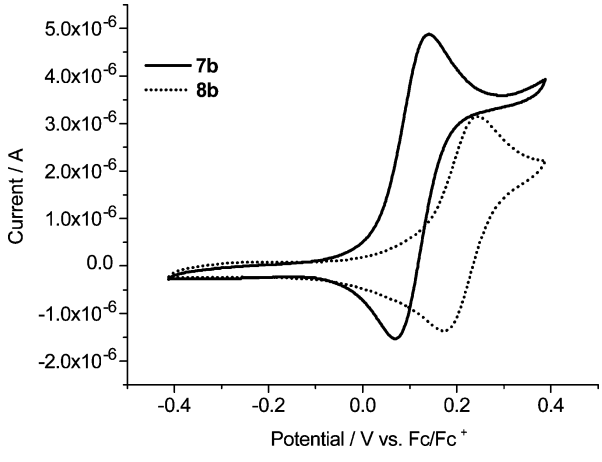


Figure 2. Cyclic voltammogram of **7b** and **8b** on a gold electrode in CH_3CN containing Bu_4NPF_6 at a scan rate of 20 mV/s.

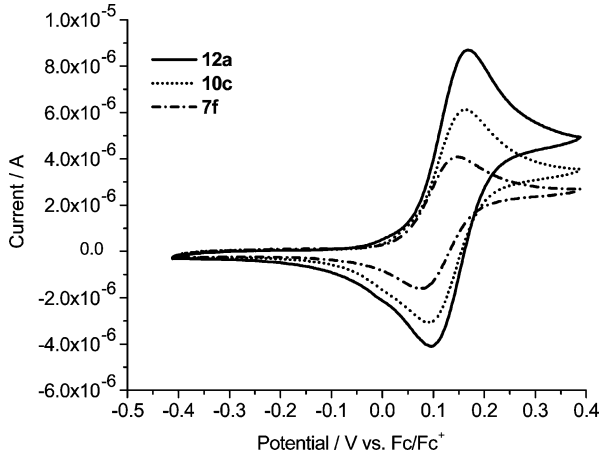


Figure 3. Cyclic voltammogram of **7f**, **10c**, **12a** on a gold electrode at a scan rate of 20 mV/s.

The final set of molecules tested by us comprises of similar dipeptide conjugates **5f** and **7g** that were synthesized via complementary strategies. The CV's (Figure 4) reveal that ferrocene conjugate **5f** has a lower redox potential than **7g** indicating the presence of higher electron density on the iron in **5f** than **7g**. The ^{13}C NMR data of these compounds also confirmed this observation. The *ipso*-C atom in **5f** gave

a signal at δ = 75 ppm in ^{13}C NMR whereas in **7g** it appeared at δ = 81 ppm again confirming the high electron density on the Cp ring in **5f** and in turn on the iron atom. The formal potentials ($E°_f$) and peak separation values ($E_p^{anodic} - E_p^{cathodic}$) for the ferrocene conjugates are listed in Table 4. The $\Delta E_{(a-c)}$ ($E_p^{anodic} - E_p^{cathodic}$) is almost the same for all the compounds (70 mV) indicating a good electrochemical reversibility under the conditions employed.

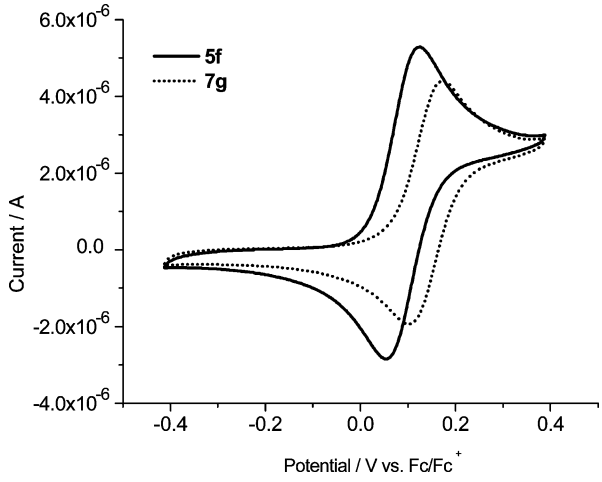


Figure 4. Cyclic voltammogramms of **5f** and **7g** on a gold electrode at a scan rate of 20 mV/s.

Table 4. $E°_f$ (midpoint of oxidation and reduction peaks) and the difference between the anodic and cathodic peak potentials $\Delta E_{(a-c)}$ for the compounds listed above.

Entry	Compd.	$E°_f$ /mV vs. Fc/Fc$^+$	ΔE_{a-c} / mV
1	**7b**	105	70
2	**8b**	209	71
3	**7f**	110	72
4	**10c**	126	71
5	**12a**	132	72
6	**5f**	89	70
7	**7g**	138	70

Conclusions

We have synthesized a number of ferrocenyl-substituted amino acids and peptides ("conjugates") employing 1,3-dipolar cacloaddition with diverse alkynes and azides derived from amino acids and peptides. Two complementary strategies were employed for their synthesis. Using ferrocene-derived bis-azide **3** conjugates containing substituents in the both rings were obtained. Novel bimetallic and trimetallic conjugates were also synthesized in very good yield. In a novel strategy, unsymmetrically substituted conjugates **17** and **18** were easily accessed. We have also shown the versatility of our methodology by synthesizing dipeptide **14c** in a divergent manner. Finally, the novel ferrocenophane **19** was synthesized from the bis-azide **3** and bis-alkyne **9b** via bimolecular condensation. A few selected ferrocene conjugates were characterized electrochemically.

Experimental Section

General Procedure for the Synthesis of Monovalent Ferrocene Conjugates 5a–f, 7a–g, 17, 18: To a well-stirred solution of azide (1 mmol) and alkyne **1** (1 mmol) in *t*BuOH/H_2O (1:1) (8 mL) $CuSO_4·5H_2O$ (0.025 g, 0.1 mmol) was added followed by sodium ascorbate (0.040 g, 0.2 mmol). The reaction mixture was stirred for respective time at room temperature. Ethyl acetate (30 mL) was added into the reaction mixture, washed with brine (15 mL) and filtered through anhydrous sodium sulfate. After evaporation of ethyl acetate, the crude product was purified by flash chromatography on silica gel.

Compound 5a: Yellow gum, yield 95% (0.448 g). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.52 (s, 1 H), 7.27–7.19 (m, 3 H), 7.0 (d, J = 6.8 Hz, 2 H), 6.5 (br. s, 1 H), 5.01 (dd, J_1 = 22.4, J_2 = 16.4 Hz, 2 H), 4.71 (dd, J_1 = 13.6, J_2 = 6.8 Hz, 1 H), 4.74 (s, 1 H), 4.71 (s, 1 H), 4.33 (s, 2 H), 4.07 (s, 5 H), 3.71 (s, 3 H), 3.14 (dd, J_1 = 14, J_2 = 8 Hz, 1 H), 3.02 (dd, J_1 = 14, J_2 = 6.8 Hz, 1 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.1, 164.9, 147.6, 135.2, 129.1, 128.7, 127.3, 120.1, 74.7, 69.6, 68.8, 66.7, 66.6, 53.3, 52.7, 52.5, 37.5 ppm. IR (neat): $\tilde{\nu}$ = 3642, 3289, 2953, 1744, 1693, 1540, 1219, 736 cm^{-1}. HRMS: *m*/*z*: calcd. for $C_{24}H_{24}FeN_4O_3$ [M + Na$^+$]: 495.1095; found 495.1084. $[a]_D^{23}$ = 35.0 (c = 2, $CHCl_3$).

General Procedure for the Synthesis of Disubstituted Ferrocene Conjugates 8a–g: To a well-stirred solution of (1,1′-ferrocenediyl)dimethyl diazide (**3**) (0.5 mmol) and amino acid derived alkyne (1 mmol) in *t*BuOH/H_2O (1:1) (8 mL) $CuSO_4·5H_2O$ (0.025 g, 0.1 mmol) was added followed by sodium ascorbate (0.040 g, 0.2 mmol). The reaction mixture was stirred for required time at room temperature. Ethyl acetate (40 mL) was added into the reaction mixture and was washed with brine (15 mL) and filtered through anhydrous sodium sulfate. After evaporation of ethyl acetate, the crude product was purified by flash chromatography on silica gel.

Compound 8a: Yellow gum, yield 90% (0.354 g). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.61 (s, 2 H), 5.82 (d, J = 6.8 Hz, 2 H), 5.19 (s, 4 H), 5.18 (s, 4 H), 4.45 (dd, J_1 = 12.8, J_2 = 7.6 Hz, 2 H), 4.24–4.21 (m, 8 H), 3.72 (s, 6 H), 2.5 (t, J = 7.2 Hz, 4 H), 2.16–1.89 (m, 10 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.3, 155.7, 143, 123.4, 82.3, 69.7, 69.6, 69.5, 58.2, 52.9, 52.4, 49.3, 31.5, 29.8, 15.3 ppm. IR (neat): $\tilde{\nu}$ = 3327, 2923, 1721, 1531, 1439, 1201, 1048 cm^{-1}. HRMS: *m*/*z*: calcd. for $C_{32}H_{42}FeN_8O_8S_2$ [M + Na$^+$]: 809.1814; found 809.1801. $[a]_D^{23}$ = 14.0 (c = 2, $CHCl_3$).

General Procedure for the Synthesis of Divalent Conjugates 10b–d Derived from Amino Acid Derived Bis-Alkynes 9b–d: To a well-stirred solution of azide **2** (1.05 mmol) and amino acid derived bis-alkyne (0.5 mmol) in *t*BuOH/H_2O (1:1) (12 mL) $CuSO_4·5H_2O$ (0.025 g) was added followed by sodium ascorbate (0.040 mg). The reaction mixture was stirred for required time. Ethyl acetate (50 mL) was added into the reaction mixture and was washed with brine (25 mL) and filtered through anhydrous sodium sulfate. After evaporation of ethyl acetate, the crude product was purified by flash chromatography on silica gel.

Compound 10b: Yellow gum, yield 88% (0.338 g). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.46 (s, 1 H), 7.38 (s, 1 H), 7.15 (d, J =

4 Hz, 2 H), 6.97–6.95 (m, 2 H), 5.27 (s, 2 H), 5.26 (s, 2 H), 5.17 (s, 2 H), 5.11 (s, 2 H), 4.56 (dd, J_1 = 13.6, J_2 = 6 Hz, 1 H), 4.28–4.16 (m, 18 H), 3.02–2.96 (m, 2 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.1, 155.3, 142.9, 141.8, 135.3, 129.1, 128.4, 126.9, 123.2, 122.9, 80.7, 80.4, 69.1, 69.0, 68.9, 68.8 (2 C), 58.4, 58.3, 54.7, 50.0, 49.9, 37.8 ppm. IR (neat): $\tilde{\nu}$ = 3420, 2926, 2248, 1722, 1515, 1049, 732 cm^{-1}. HRMS: *m*/*z*: calcd. for $C_{38}H_{37}Fe_2N_7O_4$[M + Na^+]: 790.1504; found 790.1565. $[a]_D^{23}$ = 4 (*c* = 1.0, $CHCl_3$).

General Procedure for the Synthesis of Trivalent Ferrocene Conjugates 12a, 13a: To a well-stirred solution of amino acid derived trisalkyne **12** (1 mmol) and ferrocenylmethyl azide **2** (0.795 g, 3.3 mmol) in *t*BuOH/H_2O (1:1) (14 mL) $CuSO_4$·5H_2O (0.063 g, 0.25 mmol) was added followed by sodium ascorbate (0.099 g, 0.5 mmol). The reaction mixture was stirred for 24 h. Ethyl acetate (50 mL) was added into the reaction mixture and was washed with brine (25 mL) and filtered through anhydrous sodium sulfate. After evaporation of ethyl acetate, the crude product was purified by flash chromatography on silica gel.

Compound 12a: Yellow gum, yield 85% (1.001 g). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.59 (s, 1 H), 7.47 (s, 1 H), 7.39 (s, 1 H), 6.96 (d, *J* = 8.8 Hz, 2 H), 6.92 (d, *J* = 8.4 Hz, 2 H), 5.32 (s, 2 H), 5.29 (s, 2 H), 5.26 (s, 4 H), 5.17–5.11 (m, 4 H), 4.55 (dd, J_1 = 13.5, J_2 = 5.7 Hz, 1 H), 4.28–4.16 (m, 27 H), 3.02–2.98 (m, 2 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.9, 155.3, 153.4, 149.9, 142.8, 141.7, 141.4, 133.4, 130.3, 123.4 (2 C), 122.9, 120.9, 80.7, 80.5, 80.4, 69.1 (2 C), 69.0, 68.9 (2 C), 68.8 (2 C), 61.5, 58.5, 58.3, 54.6, 50.1, 50.0, 49.9, 37.2 ppm. IR (neat): $\tilde{\nu}$ = 3343, 2935, 1734, 1515, 1246, 1048, 824, 731 cm^{-1}. HRMS: *m*/*z*: calcd. for $C_{53}H_{51}Fe_3N_{10}O_7$[M + Na^+]: 1129.1810; found 1129.1868. $[a]_D^{23}$ = –2.0 (*c* = 2, $CHCl_3$).

Electrochemical Measurements: Cyclic voltammetric experiments were carried out using EG&G 273A (PAR USA) electrochemical system. De-aerated CH_3CN containing 0.2 M Bu_4NPF_6 was used as the electrolyte. The concentration of analytes was kept at 1 mM. The working electrode was gold disc, the counter electrode was a large area Pt foil, and the reference electrode was Ag/AgCl/0.1 M Bu_4NPF_6 in acetonitrile. The working electrode was carefully polished with basic Al_2O_3/water slurry, washed with methanol and sonicated in H_2O/MeOH-CH_3CN (1:1):1 mixture for 15 min. The working electrode was subsequently dipped into hot "piranha" solution for a few seconds, washed well with doubly distilled water, followed by electrochemical cycling in 0.5 M H_2SO_4 between 0.2 V and 1.5 V vs. SCE until reproducible voltammogramms were obtained. The analytes were subsequently introduced and the voltammogramms were recorded at 20 mV/s scan rate. The reference electrode is calibrated against ferrocene/ferrocenium redox couple (Fc/Fc^+) in the same supporting electrolyte. The formal potential of ferrocene/ferrocenium redox couple is 0.412 V (vs. Ag/AgCl/0.1 M Bu_4NPF_6) in the solvent used for present studies (acetonitrile). Accordingly all the potential values in the voltammograms are given with respect to ferrocene/ferrocenium couple.

Supporting Information (see also the footnote on the first page of this article): Spectroscopic and analytical data of all the new compounds.

Acknowledgments

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