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Efficient synthesis of unsymmetrically disubstituted ferrocenes: towards electrochemical dipeptide-Fc-biosensors

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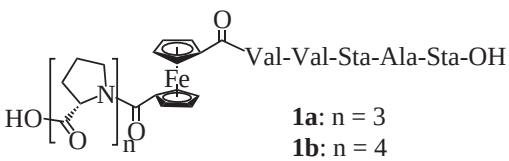
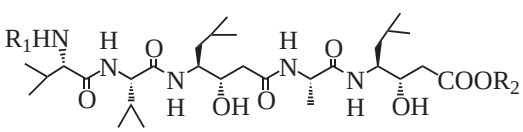
Abstract—Unsymmetrically disubstituted ferrocenes (**1a**, **1b**), electrochemical dipeptide-Fc-biosensors, were synthesized by a straightforward synthetic method from 1,1′-ferrocenedicarboxylic acid. In addition, an efficient solution synthesis of pentapeptide (**2**) was reported.

In recent years, electrochemical biosensors have been developed for the analytical determination of biological and organic analytes.[1] The research of DNA biosensors has been particularly successful,[2] providing new methods for mutation detection. This technology offers the benefits of being fast, easy-to-use, and inexpensive, as well as having a wide range of potential applications, such as the detection of diseases, monitoring air quality, and detection of bacterial contamination in food items. Thus, the development of new biosensors is presently receiving a significant amount of attention.[1,2]

Our group has been interested in the electrochemistry of ferrocenoyl (Fc) peptides and its potential application for the development of Fc-based electrochemical biosensors.[3] We discovered that the redox properties of the Fc moiety in Fc-peptides is sensitive even to small variations in peptide structure.[3] Furthermore, the oligoproline chain allows for electronic communication between the Fc group and the C-terminus of the oligopeptide chain.[4] We decided to prepare two Fc-oligoproline constructs with a second podant peptide chain able to interact strongly with aspartic proteases, such as HIV-1 protease. We designed two 1,1′-bispeptide-Fc compounds (**1a**, **1b**), in which the podant pentapeptide (**2**) is attached to the 1′-Cp ring of the Fc group. **2** has the identical peptide sequence to that found in pepstatin, a potent naturally occurring inhibitor of aspartic protease, including pepsin, HIV-1, and HIV-2 proteases,[5] but has not been prepared before. Structurally, **1a** and **1b** are unsymmetrically 1,1′-disubstituted ferrocenes. Importantly, known synthetic methodologies for the preparation of unsymmetrically 1,1′-disubstituted ferrocene derivatives involve complex transformations.[6] Here we have overcome this synthetic challenge and report the straightforward but selective introduction of two different peptides into the ferrocene framework to produce **1a** and **1b**. Furthermore, we report the efficient synthesis of pentapeptide (**2**) in solution.



1a: n = 3
1b: n = 4



pepstatin: R_1=iso-valeryl R_2=H
2: R_1=Boc R_2=Et

Keywords: ferrocene; synthesis; pepstatin; disubstitued ferrocenes; biosensors.
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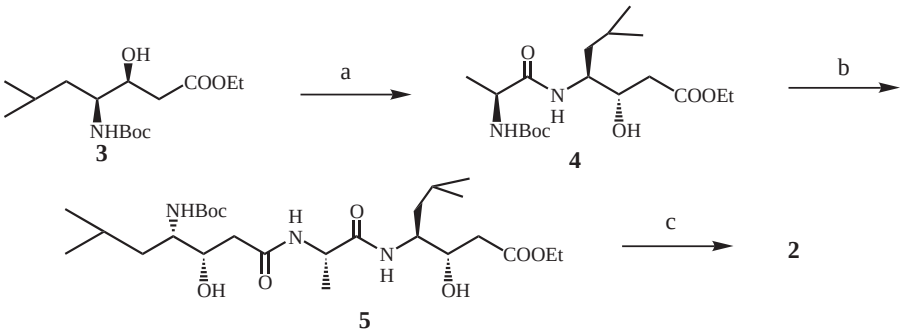


Scheme 1. Reagents and conditions. (a) i. TFA, ii. Boc-Ala-OSu, CH_2Cl_2, DMF, 84%; (b) i. TFA, ii. Boc-Sta-OSu, CH_2Cl_2, DMF, 54%; (c) i. TFA, ii. Boc-Val_2-OSu, HOBt, EDC, CH_2Cl_2, 48%.

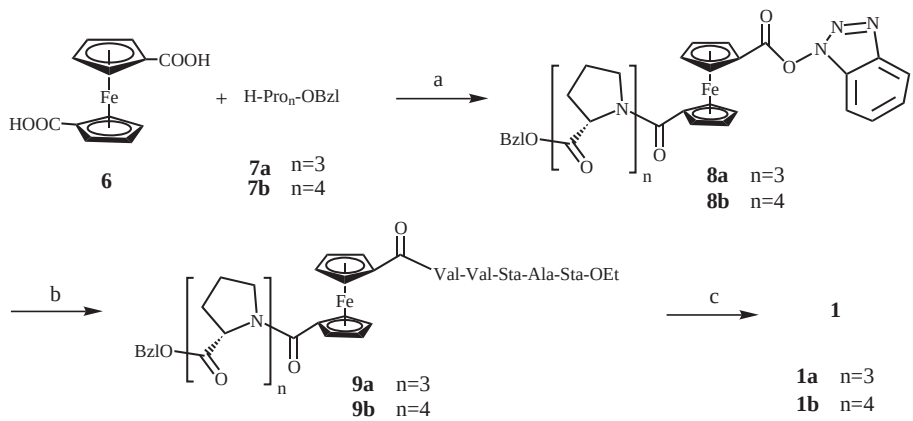
The synthesis of pentapeptide (**2**) is shown in Scheme 1. Statine (**3**) was prepared from L-lucine in four steps with an overall yield of 27% {$[\alpha]_D^{20}$ −39 (*c* 1.1, MeOH); lit.[7] $[\alpha]_D^{20}$ −37.9 (*c* 0.84, MeOH)}.[7] Commercially available Boc-Ala-OH was transformed to Boc-Ala-OSu via a reported method.[8] Treatment of Boc-Sta-OEt with trifluoroacetic acid gave H-Sta-OEt, which was coupled with Boc-Ala-OSu in a mixed solvent (CH_2Cl_2:DMF, 5:1) at room temperature to afford Boc-Ala-Sta-OEt (**4**). After deprotection of the C-terminus by base hydrolysis,[7] **3** was transformed into Boc-Sta-OSu and then coupled with dipeptide **4** to form Boc-Sta-Ala-Sta-OEt (**5**).

Employing the reported method,[9] Boc-Val_2-OMe was obtained from Boc-Val-OH and H-Val-OMe. Deprotection of the methyl group with NaOH in dioxane/H_2O afforded Boc-Val_2-OH. In the last step of the peptide coupling sequence, Boc-Val_2-OH and tripeptide **5** were coupled together by the standard carbodiimide protocol[10] in CH_2Cl_2 at room temperature for 20 h to give pentapeptide (**2**) {mp 188–190°C, $[\alpha]_D^{20}$ −60.1 (*c* 1.0, $CHCl_3$)}. The product was purified by column chromatography on silica gel (CH_2Cl_2–MeOH, 98:2).

In our previous research on syntheses of bisoligoproline ferrocenes, we isolated 1-oligoproline-1′-OBt-ferrocene derivatives (**8a**, **8b**).[11] These complexes are air and moisture stable and easily separated from the symmetric 1,1′-bisoligoproline-ferrocenes by chromatography on silica gel. Since the active OBt-ester can be readily exchanged for another peptide using the established procedures,[10] both **8a** and **8b** are very useful synthons for ferrocene derivatives having two different peptide chain and are readily prepared from 1,1′-ferrocenedicarboxylic acid. Compounds **8a** and **8b** were obtained by treatment of 1.5 molar equivalents of oligoprolines with 1 mol of 1,1′-ferrocenedicarboxylic acid in the presence of *N*-hydroxybenzotriazolw (HOBt) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) in CH_2Cl_2 at room temperature (yield: 55% for **8a**, 45% for **8b**) (Scheme 2).

As outlined in Scheme 2, the N-terminal of pentapeptide (**2**) was deprotected to give H-Val_2-Sta-Ala-Sta-OEt, which was then successfully coupled with **8a** and **8b** in CH_2Cl_2 at room temperature to give **9a** and **9b** in yields of 59 and 55%, respectively.[12] Finally after deprotection of the benzyl and ethyl groups in a basic dioxane–water mixture, the target compounds **1a** and **1b** were obtained.[13]

In summary, we report a solution synthesis of pentapeptide (**2**) which has the identical peptide sequence to that in pepstatin. Furthermore, we report a straightforward and useful synthetic method for the preparation of unsymmetrically 1,1′-disubstituted ferrocenes from 1,1′-ferrocenedicarboxylic acid. In this work, we synthesized two novel 1,1′-dipeptide-ferrocenes (**1a**, **1b**). Currently work is in progress to evaluate the electro-



Scheme 2. Reagents and conditions. (a) HOBt, EDC, CH_2Cl_2; (b) H-Val_2-Sta-Ala-Sta-OEt, CH_2Cl_2; (c) NaOH, dioxane/H_2O.

chemistry of **1a** and **1b** and their ability to bind to aspartate proteases.

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12. Compounds **9a** and **9b** were purified by column chromatography on silica gel (CH_2Cl_2–MeOH, 96:4). Selected data for **9a** and **9b**. **9a**: mp 129–130°C, $[\alpha]_D^{20}$ −46 (*c* 0.91, $CHCl_3$), HRMS (FAB) *m*/*z* calcd for $C_{65}H_{95}FeN_8O_{14}$ (M^++1) 1267.6317, found 1267.6324. **9b**: mp 131–132°C, $[\alpha]_D^{20}$ −41 (*c* 1.3, $CHCl_3$), HRMS (FAB) *m*/*z* calcd for $C_{70}H_{102}FeN_9O_{15}$ (M^++1) 1364.6845, found 1364.6840.
13. Pure **1a** and **1b** were obtained in yields of 63 and 70%, respectively, by recrystallization from MeOH–Et_2O. Selected data for **1a** and **1b**. **1a**: mp 155–157°C, $[\alpha]_D^{20}$ −69 (*c* 1.0, MeOH), HRMS (FAB) *m*/*z* calcd for $C_{56}H_{85}FeN_8O_{14}$ (M^++1) 1149.5535, found 1149.5522. **1b**: mp 172–173°C, $[\alpha]_D^{20}$ −89 (*c* 1.1, MeOH), HRMS (FAB) *m*/*z* calcd for $C_{61}H_{92}FeN_9O_{15}$ (M^++1) 1246.6062, found 1246.6157.