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Ferrocene derivatives supported on poly(*N*-vinylpyrrolidin-2-one) (PVP): Synthesis of new water-soluble electrochemically active probes for biomolecules

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Dedicated to Professor Stefano Maiorana on the occasion of his 70th birthday.

Abstract

Carboxy-terminated polyvinylpyrrolidin-2-one (PVP) has been used as a new water-soluble and biocompatible polymeric support for a series of ferrocene labeled amino acid and peptide nucleic acid (PNA) monomer derivatives **4**–**7**. The organometallic polymer-conjugates thus obtained are new and potentially useful as water-soluble electrochemically active probes for biomolecules. In view of such application, their electrochemical activity has been evaluated and has proved very high notwithstanding the complexity and bulkiness of the molecule, affording detection limits down to 10^{-8} M in the aqueous medium.

Keywords: Bioorganometallic chemistry; Ferrocene; Functionalized PVP; Electrochemically active probes; Polymer conjugation

1. Introduction

Soluble polymers are of great importance for industrial applications and in biological systems [1,2]; for example the conjugation of biologically active molecules to water-soluble polymers [3] is a promising strategy for improving their pharmacological and pharmacokinetic profile and therapeutic index [4]. A number of soluble polymers having linear, hyperbranched or dendritic structures have been tested for the covalent conjugation of low and high molecular weight bioactive molecules [5] constituting the so-called polymer therapeutics. Although many efforts are being made to develop novel polymeric carriers, synthetic polymers that have been tested in clinically evaluated drug conjugates have been mainly restricted to poly(glutamic acid) (PG), *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymers, and polyethylene glycol (PEG). PEG conjugation [6] of therapeutically relevant proteins is a well-established technology and a number of PEG-ylated proteins have received market approval or are in advanced clinical trials. Within the class of biocompatible soluble polymers another well-known polymeric agent, namely poly(*N*-vinylpyrrolidin-2-one) (PVP) [7–10] **1** (Fig. 1), might be a promising candidate for polymer therapeutics. In fact, PVP is a water-soluble, biocompatible, amphiphilic polymer with a huge number of relevant chemical and biological properties [11–14]. However, the absence of reactive functional groups on the polymer chain prevents its extensive use for covalent drug conjugation, thus making the functionalization of PVP a relevant synthetic target in polymer chemistry.

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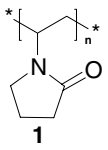

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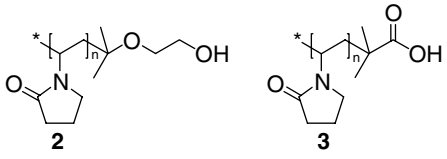
Fig. 1. Poly(*N*-vinylpyrrolidin-2-one) (PVP).

The synthesis and characterization of some end- [15–19] and lactam ring- [20] functionalized PVP have been recently reported. For example, PVP oligomers bearing hydroxy [15] or carboxy [18] groups at one chain end, such as **2** (PVP–OH) and **3** (PVP–COOH) (Fig. 2), have been prepared by the radical polymerization of 1-vinylpyrrolidin-2-one (VP) in the presence of suitable chain transfer agents. These polymers have been used in the synthesis of copolymers [21] and for the modification of bioactive molecules or macromolecules [22,23].

The study of further applications of functionalized PVP, as new water-soluble supports for bioactive and organic molecules, is certainly an important research field and we considered the PVP–COOH **3** [18] as the most useful derivative to this end. In fact, we found that the carboxy group of **3** can be reacted directly with an amino or hydroxy group of a drug, thereby directly forming a conjugate [24] (Scheme 1, path a). On the other hand, PVP–COOH **3** can serve as a convenient starting material for preparing other functionalized polymers and we were able to obtain a variety of PVP derivatives (Scheme 1, path b) arising from the condensation of the carboxy group of **3** with the amino group of different bifunctional reagents [25]. These new PVP oligomers are very useful to enlarge the number of the molecules that can be conjugated and the kind of linkers connecting them to the support.

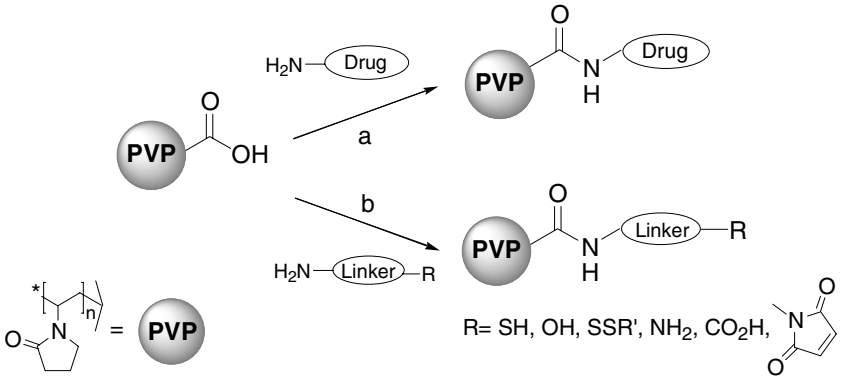


2 3

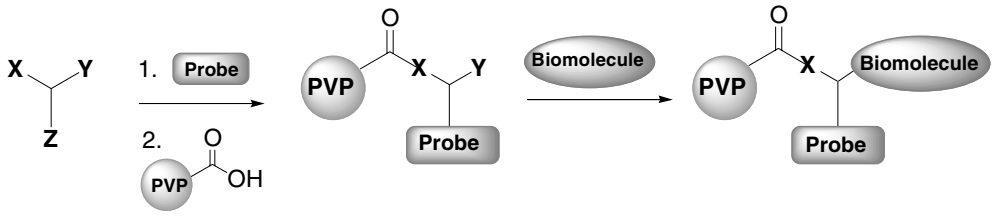
Fig. 2. Hydroxy-(PVP–OH) **2** and carboxy-(PVP–COOH) **3** end-functionalized PVP.

In view of diagnostic applications of PVP bioconjugates, the possibility of inserting an analytically detectable probe on the polymer might represent an important step in developing this chemistry. But, since only mono-functionalized PVP can be obtained by chain-transfer radical polymerization, it is necessary to bind, at the end of the chain, a trifunctional molecule able to link the probe, the polymer and the biomolecule (Scheme 2). Some ω-functionalized amino acids (*i.e.* tyrosine, lysine and serine) are suitable candidates to this aim, and in principle, after the linkage to some fluorescent or electrochemically active molecules, their conjugation to PVP could provide new water-soluble analytical probes, a quite interesting feature for application in the biomedical field [26].

In this paper, we report our results concerning the covalent support on PVP of mono- and multi-ferrocene labeled amino acid and PNA monomer derivatives, as well as the investigation of the electrochemical behaviour of the corresponding water-soluble polymers thus obtained.



Scheme 1. Conjugation of molecules to PVP–COOH.



Scheme 2. Probe-containing PVP.

2. Results and discussion

The first molecules of choice are shown in Fig. 3, and are constituted by mono- and tri-ferrocene labeled tyrosine derivatives **4** and **5**.

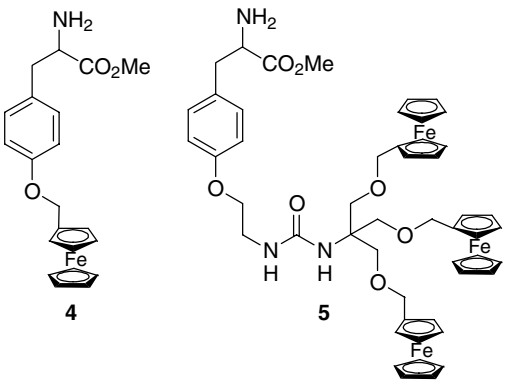


Fig. 3. Ferrocene labeled tyrosine.

In addition, the PVP conjugation of the previously synthesized and electrochemically investigated [27] mono- and tri-ferrocene labeled tyrosine Peptide Nucleic Acid (PNA) monomers **6** and **7** (Fig. 4), was investigated.

PNA oligomers [28], are synthetic pseudo-peptide mimics of DNA, have great potential for DNA recognition as new therapeutics in anti-sense and anti-gene strategies and are useful for diagnostic applications. However, a rapid progress in extensive use of PNA is restrained by its poor water solubility and cell permeability. The possibility of conjugating PNA to PVP might be a promising approach to solve this problem, especially in the presence of a probe allowing detection of its cellular uptake.

Mono-ferrocene labeled tyrosine **4**, was prepared as previously reported [27d], while tri-ferrocene tyrosine **5** was synthesized as described in Scheme 3: *N*-Cbz tyrosine methyl ester **8** was reacted with 2-(Boc-amino)ethyl bromide **9**, in the presence of cesium carbonate and KI in DMF at room temperature, affording compound **10** in 70% yield. After the cleavage of *N*-Boc protection, derivative **11** was condensed with tris-ferrocene isocyanate **12**

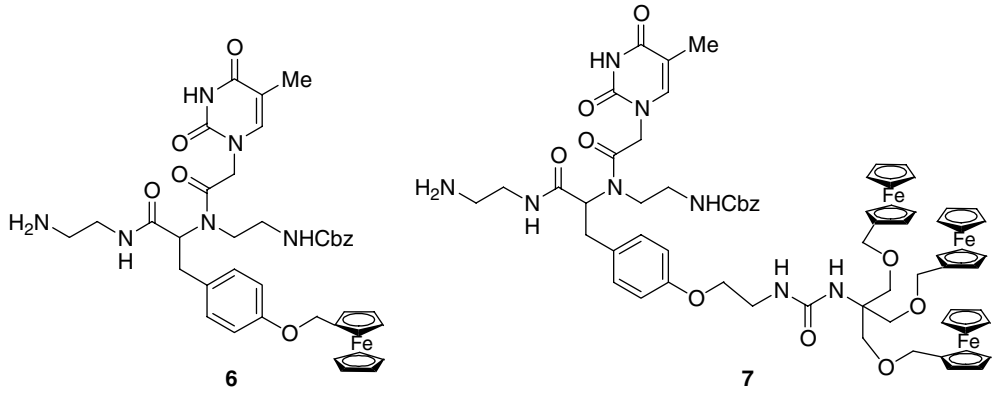
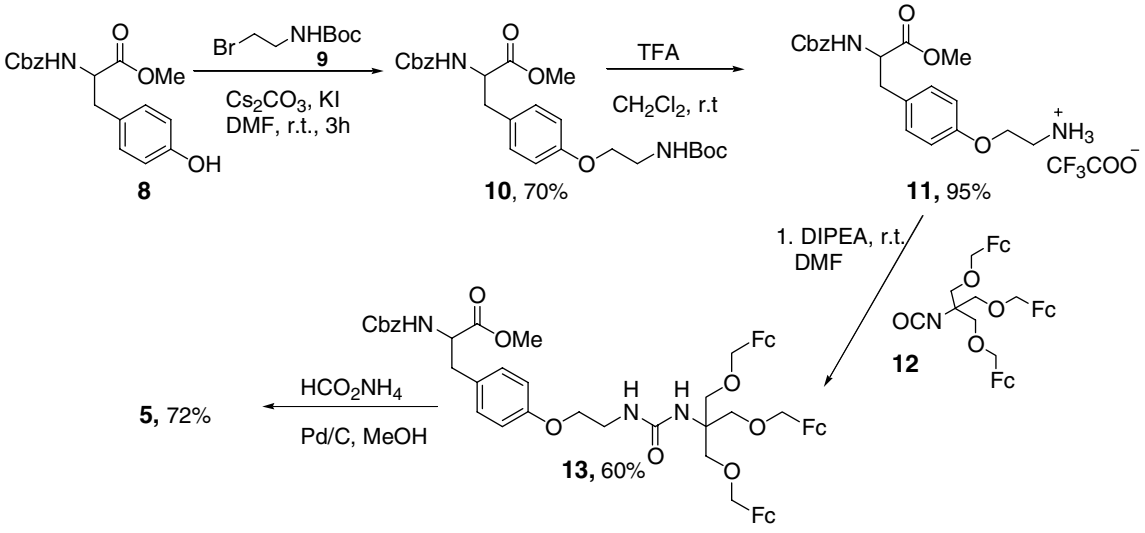


Fig. 4. Ferrocene-labeled PNA monomers.



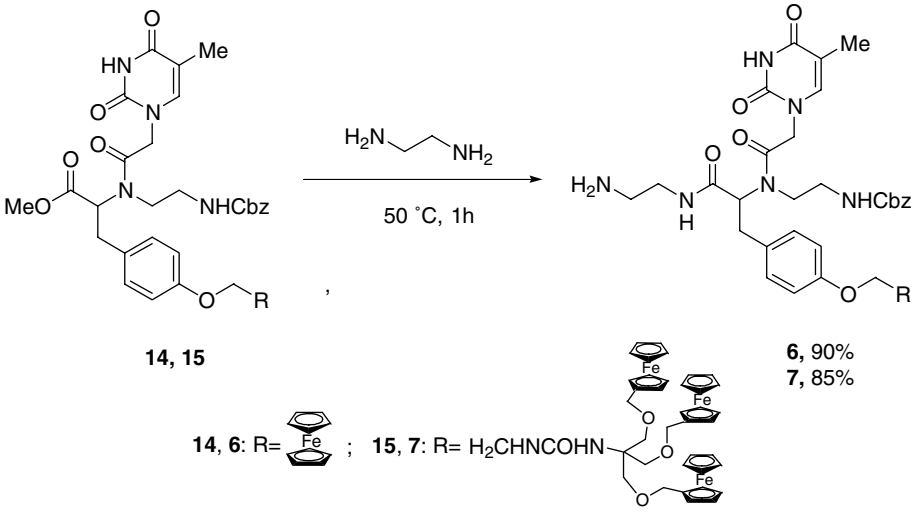
Scheme 3. Synthesis of tri-ferrocene labeled tyrosine **5**.

[27a] to give **13**. Tri-ferrocene labeled tyrosine **5** was eventually obtained in 72% yield, after the cleavage of Cbz group in the presence of Pd/C and ammonium formate.

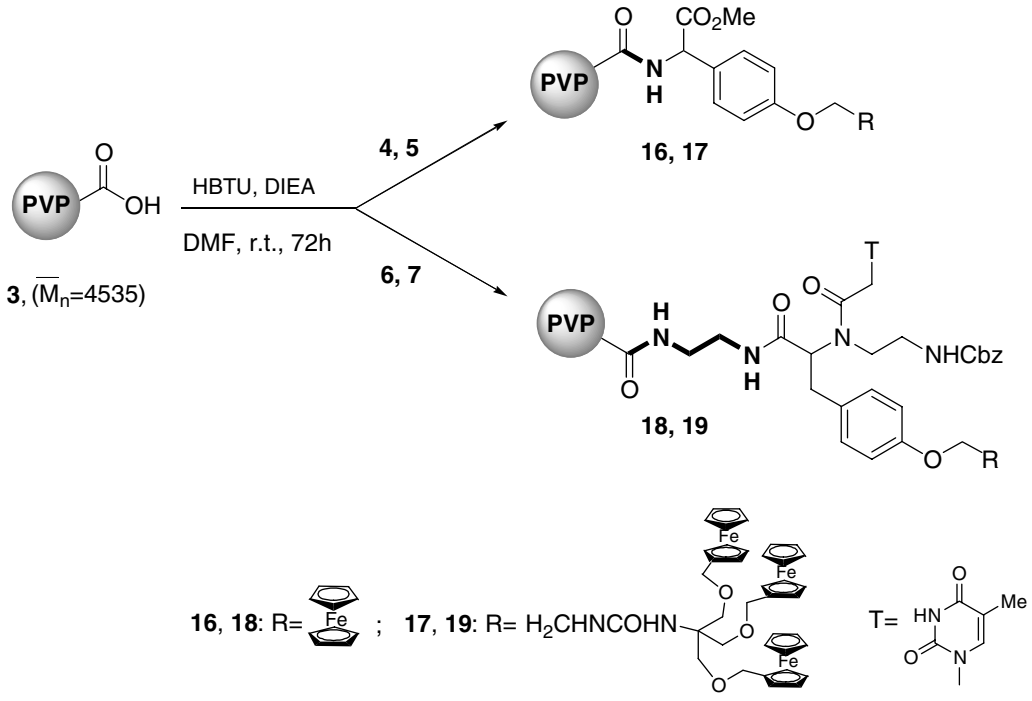
PNA monomers **6** and **7**, bearing a 2-aminoethyl amide group, were obtained in good yields from the corresponding methyl ester monomers **14** and **15** heated at 50 °C in ethylendiamine solvent (Scheme 4).

The condensation of PVP–COOH with compounds **4**–**7** was run in DMF using *O*-(Benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) in the presence of diisopropylethyl amine (DIEA) as a condensing agent and stirring the mixture at room temperature for 3 days (Scheme 5). The PVP conjugated **16**–**19** thus obtained were isolated, after the evaporation of the solvent, by precipitation from diethyl ether. The polymers were then dissolved in water (in which they are fully soluble) and purified by ultrafiltration through a membrane with a nominal cut-off of 3000. After lyophilization, polymers **16**–**19** were isolated as a light brown powder and completely characterized.

It is worth emphasizing that in the case of tyrosine derivatives **16** and **17** the amide bond, formed with the α-nitrogen atom, directly connects the amino acid to the polymer, while a short amino-ethyl linker is present between PVP



Scheme 4. Synthesis of PNA monomers **6** and **7**.



Scheme 5. PVP conjugation of the ferrocene derivatives **4**–**7**.

and PNA monomers in **18** and **19**, keeping the organometallic moiety a little further from the core of the polymer (Scheme 5).

In the perspective of using such PVP conjugates as active water-soluble probes it is important to evaluate how the presence of the polymer affects their electrochemical activity.

In this context, a voltammetric investigation on the PVP-conjugated **16**–**19** using differential pulse voltammetry (DPV) and square wave voltammetry (SWV) has been carried out. Fig. 5 summarizes the DPV curves of the investigated compounds, at 2×10^{-4} M concentration in water. The peak potentials against the $Me_{10}Fc^{+}|Me_{10}Fc$ (decamethylferricinium|decamethylferrocene) reference redox couple are at 0.463, 0.395, 0.468 and 0.439 V for compounds **16**, **17**, **18**, and **19**, respectively. The large difference in the redox potential between compound **17** and the others might be connected with a different accessibility of the active site and/or a locally modified solvental environment for this compound. In order to better clarify this difference, a complete cyclovoltammetric characterization in different solvents accompanied by theoretical molecular modeling studies, is currently in progress. The results show a good voltammetric response in terms of current intensity and tripling of the current in a single oxidation wave in the case of the conjugates with three ferrocenes **17** and**19**. This observation points to the three ferrocene groups being independent [27c]. Moreover, comparing the peak currents of the PNA monomers **18**, **19** vs. tyrosine derivatives **16**, **17**, either mono- or tri-functionalized (recorded both in CV and DPV mode), the smaller tyrosine derivatives show lower currents with respect to the bulkier PNA ones. At constant concentration, reaction mechanism and number of transferred electrons, normally higher currents are due

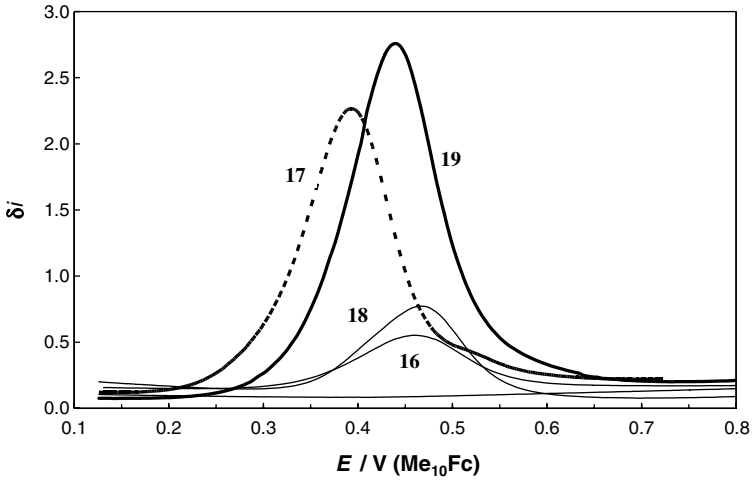


Fig. 5. Differential pulse voltammograms of PVP conjugated compounds at 2×10^{-4} M concentration in H_2O (**16**, thin line; **17**, thick line; **18**, thin plain line; **19**, thick plain line).

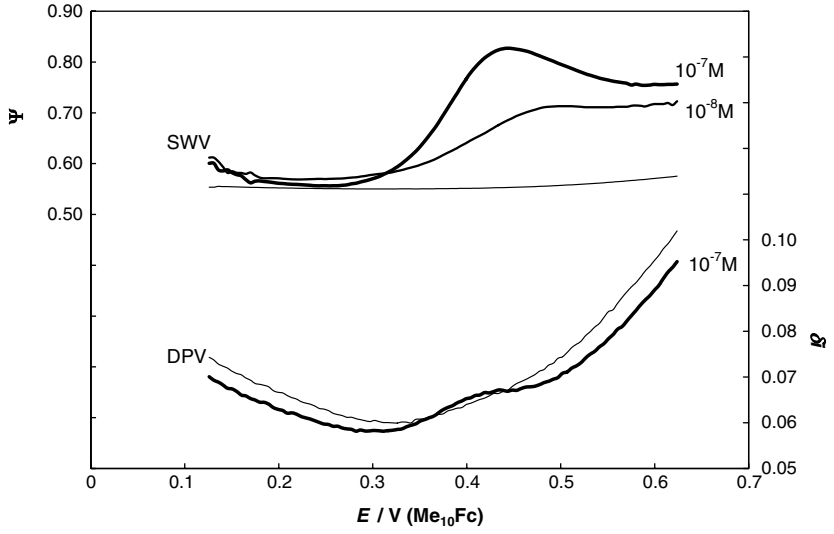


Fig. 6. Differential pulse (DPV) and square wave (SWV) voltammograms of compound **19** at 10^{-7}–10^{-8} M concentration in H_2O (thin line: background).

to higher diffusion coefficients and/or higher accessibility of the active site. Here such desirable conditions appear to be better complied with in the case of the bulkier PNA fragment **18** and **19**.

The interesting and promising results have prompted us to investigate on the aqueous detection limit for compound **19**. Actually, **19** shows (Fig. 6) an appreciable response down to 10^{-7} M and even 10^{-8} M, using the SWV, notwithstanding the complexity and bulkiness of the molecule, and in the absence of any amplification step.

3. Conclusions

We have investigated the application of poly(vinylpyrrolidin-2-one) (PVP), as a new water-soluble and biocompatible polymeric support in the field of bioorganometallic chemistry [29]. A series of mono and tri-ferrocene tyrosine and peptide nucleic acid (PNA) monomer derivatives **4**–**7**, have been supported on carboxy-terminated PVP affording new and potentially useful water-soluble electrochemical probes for biomolecules. In view of such application their electrochemical activity has proved to be very high, which is surprising given the complexity and bulkiness of the molecule, affording detection limits down to 10^{-8} M in aqueous media.

4. Experimental

4.1. Materials and methods

All the chemicals used (laboratory or analytical grade) were purchased from Sigma–Aldrich or Fluka unless otherwise indicated. All the reactions were performed under nitrogen atmosphere using a standard vacuum line. The reactions were monitored by TLC, carried out on Kieselgel 60 F_{254} plates (Fluka), with detection by UV, $KMnO_4$ or developing in I_2 chamber. Merck silica gel 60 (70–230 mesh) was used for column chromatography purification. ^1H and ^{13}C NMR spectra were recorded on a Bruker, AC300 and AMX 300 MHz instruments. IR spectra were recorded on a Perkin–Elmer 1725X FT-IR. Melting points were measured with a Büchi B-540 apparatus and are uncorrected. MS data were obtained by using a VG 70 EQ Micromass or a Thermo-Finningam mass spectrometer. PVP–COOH **3** [18] ($M_n = 4535$) was dried at 60 °C under vacuum for 5 h before use. Compound **4**, **8** [27d] and **12** [27a] were prepared as previously reported.

4.2. Synthesis of compound *10* [27a]

2-(Boc-amino)ethyl bromide **9** (1 mmol), Cs_2CO_3 (3 mmol) and KI (0.1 mmol) were added to a solution of (*L*)-**8** (1 mmol) in DMF (8 ml). The reaction mixture was stirred at room temperature for 3 h, diluted with water (50 ml) and extracted with EtOAc (4 × 25 ml). The combined organic layers were washed with water (30 ml), dried over Na_2SO_4, filtered and evaporated. The crude product was purified by column chromatography (light petroleum/EtOAc, 3:2; $R_f = 0.55$), obtaining **10** as a white solid in 66% yield.

(*L*)–**10**: m.p. 79–81 °C (pentane); ^1H NMR ($CDCl_3$), δ: 1.44 (s, 9H, Bu^t); 3.09–2.97 (m, 2H, CH_2Ph); 3.45–3.53 (m, 2H, CH_2NHBoc); 3.71 (s, 3H, OCH_3); 3.96 (t, 2H, CH_2O, $J = 5.1$ Hz); 4.62–4.59 (m, 1H, CH); 4.98–5.29 (m, 4H, CH_2-Cbz+NH-Cbz+NH-Boc); 6.78 (d, 2H, PhO, $J = 8.5$ Hz); 6.98 (d, 2H, PhO, $J = 8.5$ Hz); 7.33–7.35 (bs, 5H, Ph). ^{13}C NMR ($CDCl_3$, δ): 28.2, 37.0, 39.9, 52.0, 54.8, 66.6–66.8, 79.4, 114.3, 127.9–128.5, 130.1, 136.1, 155.5, 155.8, 157.4, 171.9. IR ($CHCl_3$, ν, cm^{-1}) : 1708–1718 (NHCO). MS (ESI) m/z 495.3 $(M + Na)^+$.

4.3. Synthesis of compound *11*

At room temperature TFA (3 ml, 41 mmol) was added to a solution of **10** (1.15 g, 2.43 mmol) in CH_2Cl_2 (5 ml) and the mixture stirred for 4 days. The solvent was evaporated and the residue was purified by column chromatography (EtOAc/MeOH, 9:1 $R_f = 0.2$) to obtain **11** (trifluoroacetate salt) in 95% yield.

(*L*)–**11**: Pinkish white sticky solid, ^1H NMR (CD_3OD), δ: 3.0–3.11 (m, 2H, CH_2Ph); 3.19–3.23 (m, 2H, $CH_2NH_3^+$); 3.68 (s, 3H, OCH_3); 4.17 (t, 2H, CH_2O, $J = 5.1$ Hz); 4.36–4.44 (m, 1H, CH); 5.02 (s, 2H, CH_2-Cbz); 6.87 (d, 2H, PhO, $J = 9$ Hz); 7.11 (d, 2H, PhO, $J = 9$ Hz); 7.29–7.31 (m, 5H, Ph Cbz). ^{19}F NMR (CD_3OD, δ): −73.47. ^{13}C NMR (CD_3OD, δ): 37.78, 41.04, 52.67, 57.13, 67.56–67.65, 115.67, 128.65–128.95, 129.42, 130.92, 131.35, 158.82, 173.97. MS (ESI) m/z 373.3 (M + 1); 395.2 $(M + Na)^+$. IR (nujol, ν, cm^{-1}): 1687.9 (COOMe).

4.4. Synthesis of compound *13*

A solution of **12** (0.07 mmol) in DMF (1 ml) was added, at room temperature, to a solution of **11** (0.08 mmol) in DMF (1 ml), followed by the addition of DIPEA (0.015 mmol). The reaction mixture was stirred for 2 days, then diluted with CH_2Cl_2 (10 ml), washed with H_2O (2 × 10 ml) and then with a 0.3 M solution of $KHSO_4$ (2 × 10 ml). The aqueous phase was then extracted with CH_2Cl_2 (2 × 10 ml); the combined organic layer was dried over Na_2SO_4, filtered and evaporated. The crude product was then purified by a column chromatography (EtOAc/ETP, 4:6) to get **13** as orange yellow oil in 60% yield.

(*L*)–**13**: ^1H NMR ($CDCl_3$) δ: 3.5–3.72 (m, 13H, $CH_2O + CH_2Tyr + CH_2NH + COOMe$); 3.8–4.3 (m, 35H, $OCH_2Fc + Fc + CH_2O$); 4.68–4.70 (m, 1H, CH); 5.1 (s, 2H, CH_2-Cbz); 6.79 (d, 2H, PhO, $J = 8.0$ Hz); 7.01 (d, 2H, PhO, $J = 8.0$ Hz); 7.26–7.34 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$, δ): 35.7, 41.1, 51.8, 55.4, 60, 65.4, 66.3, 67.4, 67.8, 68.2, 68.8, 68.9, 83, 121.3, 127.5, 127.7, 128.3, 129.7, 135, 138, 150, 170. IR ($CHCl_3$, ν, cm^{-1}): 1723 (COOMe). MS (ESI) m/z 1113.7 (M^+); 1136.7 $(M + Na)^+$. Elemental analysis calc. (%) for $C_{58}H_{63}Fe_3$-

N_3O_9: C, 62.55; H, 5.70; N, 3.77. Found: C, 62.49; H, 5.72; N, 3.75%.

4.5. Synthesis of compound 5

Ammonium formate (0.25 mmol) and 10% Pd/C (0.02 equiv.) were added to a solution of **13** (21 mg, 0.05 mmol) in MeOH:CH_2Cl_2, 1:1 (3 ml). The reaction mixture was stirred at room temperature for 24 h and filtered through a thin pad of Celite®. The solvent was evaporated; the residue was dissolved in CH_2Cl_2 (10 ml) and washed with brine (2 × 5 ml). The aqueous phase was extracted with CH_2Cl_2 (3 × 10 ml) and the combined organic phases were dried over Na_2SO_4, filtered and evaporated to get an orange–yellow oil in 72% yield.

(L)–**5**: ^{1}H NMR (CD_3OD), δ: 3.68–2.9 (m, 13H, $CH_2O + CH_2Tyr + CH_2NH + COOMe$); 4.43–4.0 (m, 35H, $OCH_2Fc + Fc + CH_2O$); 4.68–4.52 (m, 1H, CH); 6.91 (d, 2H, PhO, $J = 9.0$ Hz); 7.14 (d, 2H, PhO, $J = 9.0$ Hz). ^{13}C NMR (CD_3OD, δ): 35.3, 40.8, 51.5, 54.9, 60.8, 66.8, 67.1, 67.6, 68.5, 68.9, 69.2, 83.5, 121.6, 129.9, 135.6, 150.4, 172.0. MS (ESI) m/z 979.7 (M + 1). IR (nujol, ν, cm^{-1}): 1687.9 (COOMe). Elemental analysis calc. (%) for $C_{50}H_{57}Fe_3N_3O_7$: C, 61.31; H, 5.87; N, 4.29. Found: C, 61.02; H, 5.89; N, 4.31%.

4.6. Synthesis of PNA monomers 6 and 7

A solution of **6** or **7** (0.2 mmol) in CH_2Cl_2 (1 ml) was slowly added into ethylendiamine (6 ml) at 50 °C. The reaction, stirred at the same temperature and monitored by TLC (eluent: ethyl acetate, $R_f = 0.03$), was complete 1 h and 30 min. After evaporation of the solvent, the residue was taken up with CH_2Cl_2 (10 ml), washed with brine (2 × 10 ml) and the aqueous phase was extracted with CH_2Cl_2 (3 × 10 ml). The combined organic phases were washed with water (3 × 10 ml), dried over Na_2SO_4 and evaporated in vacuum affording compound **6** or **7**.

6: Yellow powder. Yield = 91%. m.p. (pentane) 188–189 °C. ^{1}H NMR ($CDCl_3$), δ: 1.81 (s, 3H, CH_3); 2.79–3.3 (m, 11H, $CH + CH_2NH_2 + CH_2NHCO + CH_2PhO + CH_2NCO + CH_2NHCbz$); 4.17– 4.3 (m, 9H, Fc); 4.75 (s, 2H, OCH_2Fc); 5.05 (s, 2H, CH_2Cbz); 6.86 (m, 3H, PhO + CH=); 7.06 (d, 2H, PhO, $J = 9.0$ Hz); 7.25–7.33 (m, 5H, Cbz). ^{13}C NMR ($CDCl_3$, δ): 12.2, 32.9, 39.4, 40.4, 45.6, 49.3, 66.6, 68.5, 69.0, 69.2, 82.4, 110.4, 115.1, 127.9, 128.1, 129.1, 129.9–130.0, 141.0, 151.3, 157.8, 164.7, 168.4, 170.4. MS (ESI) m/z 765.2 (M + 1). IR (nujol, ν, cm^{-1}): 1665.5 (CONH). Elemental analysis calc. (%) for $C_{37}H_{39}FeN_5O_7$: C, 61.59; H, 5.45; N, 9.71. Found: C, 61.72; H, 5.44; N, 9.69%.

7: Dark brown sticky oil. Yield 90%. ^{1}H NMR (CD_3OD), δ: 1.89 (m, 3H, CH_3); 2.30–3.70 (m, 18H, $CH_2N + CH_2PhO + CH_2NHCbz + CH_2NHCO + CH_2NH_2 + CH_2NHCONH + CH_2O$); 3.73–4.80 (m, 38H, $CH + CH_2CO + CH_2O + Fc + CH_2Fc$); 5.1 (s, 2H, CH_2Cbz); 6.80–6.90 (m, 3H, PhO + CH=); 7.1–7.2 (d, 2H, PhO, $J = 8.5$ Hz); 7.34–7.36 (m, 5H, Cbz). ^{13}C NMR (CD_3OD, δ): 12.2, 33.2, 38.4, 39.2, 44.7, 49.0, 59.0, 63.4, 67.3, 69.2, 69.5, 78.2, 83.6, 114.5, 127.7–128.1, 130.0, 130.3, 142.2, 157.8, 158.9, 168.5. MS (ESI) m/z: 1351.5 (M + 1); 1373.5 $(M + Na)^+$. IR (nujol, ν, cm^{-1}): 1665.5 (CONH). Elemental analysis calc. (%) for $C_{66}H_{73}Fe_3N_7O_{11}$: C, 60.61; H, 5.63; N, 7.50. Found: C, 60.92; H, 5.64; N, 7.49%.

4.7. Synthesis of PVP conjugates: general procedure

HBTU (0.3 mmol) and DIEA (0.6 mmol) were added to a solution of PVP–COOH **3** (0.06 mmol) in dry DMF (2 ml). The mixture was stirred for 5 min at room temperature then a solution of **4** (0.3 mmol) in DMF (1 ml) was added. The reaction was stirred for 3 days at room temperature shielded from light, then the solvent evaporated. The residue was taken up in CH_2Cl_2 (2 ml) and the solution was dropped, under stirring, to Et_2O (100 ml). After decantation of the solvent, the brown precipitate was dissolved in water (40 ml), and purified by ultra-filtration through a membrane with a nominal cut off of 3000 (four times). Products **14**–**17** were isolated as slightly brown powders after lyophilization.

NMR characterization: In general NMR spectra of PVP derivatives show many overlapping and broad signals between 1.4 and 4.0 ppm due to CH_2 and CH signals of the polymer atactic main chain and of the pyrrolidone ring.

Compound **16**: ^{1}H NMR ($CDCl_3$) δ: 1.46–1.73 (m, CH_2); 2.02 (m, CH_2 pyr); 2.21–2.34 (m, CH_2CO); 3.18 (m, CH_2N); 3.67–3.91 (m, CH); 4.14–4.28 (m, Fc); 4.72–4.73 (m, OCH_2Fc); 6.84 (m, PhO); 6.97 (m, PhO). ^{13}C NMR ($CDCl_3$, δ): 18.3, 31.5, 35.0, 42.1, 43.7, 45.0, 66.6–69.1, 115.4, 130.3, 175.5.

Compound **17**: ^{1}H NMR ($CDCl_3$) δ: 1.46–1.73 (m, CH_2); 2.07 (m, CH_2 pyr); 2.20– 2.40 (m, CH_2CO); 3.25 (m, CH_2N); 3.62–3.94 (m, CH); 4.12–4.30 (m, Fc); 6.83 (m, PhO); 6.95 (m, PhO). ^{13}C NMR ($CDCl_3$, δ): 18.3, 31.5, 34.7, 41.8, 43.7, 44.5–44.9, 68.4, 69.1–69.6, 114.6–115.0, 130.3, 151.7, 175.3.

Compound **18**: ^{1}H NMR ($CDCl_3$) δ: 1.42–1.69 (m, CH_2); 2.02 (m, CH_2 pyr); 2.22–2.35 (m, CH_2CO); 3.20 (m, CH_2N); 3.62–3.88 (m, CH); 4.18 (m, Fc); 5.11 (m, CH_2Cbz); 6.83 (m, PhO); 6.95 (m, PhO), 7.30 (m, Cbz). ^{13}C NMR ($CDCl_3$) δ: 18.2, 31.3, 34.6, 42.0, 43.6, 44.8, 115.1, 128.2– 130.0, 175.3

Compound **19**: ^{1}H NMR (CD_3OD) δ: 1.3–1.8 (m, CH_2); 2.15 (m, CH_2 pyr); 2.25–2.5 (m, CH_2CO); 3.25 (m, CH_2N); 3.9–3.6 (m, CH); 4.0–4.3 (m, Fc); 6.8–6.9 (m, PhO); 7.1–7.2 (m, PhO); 7.3–7.4 (m, Cbz). ^{13}C NMR (CD_3OD, δ): 18.9, 32.5, 33.9, 35.9, 43.4, 45.5, 69.3–69.5, 70.2–70.8, 116.0,129.6, 155.4, 177.8.

4.8. Electrochemical measurements

The preliminary cyclovoltammetric (CV) screening and the following differential pulse voltammetry (DPV) and

square wave voltammetry (SWV) characterizations of the PVP conjugated polymers **16–19** were carried out using an Autolab PGSTAT 12 potentiostat/galvanostat (EcoChemie, The Netherlands) run by a PC with GPES software, with the following set of experimental parameters: 0.002 s modulation time; 0.1 s interval time; 0.005 V step potential; 0.025 V modulation amplitude, for DPV, and 1000 Hz frequency; 0.005 V step potential, 0.025 V amplitude, in the case of SWV.

The solutions of the four PVP conjugates (2×10^{-4} M) were prepared in highly deionized water (Millipore Milli-Q® system), with 0.1 M sodium perchlorate ($NaClO_4$, Sigma–Aldrich, 98+%) as supporting electrolyte. The working electrode was a glassy carbon GC (Amel, surface 0.071 cm^2), the counter electrode was a platinum wire, while the operating reference electrode was an aqueous saturated calomel electrode (SCE). The data have been subsequently referred to the $Me_{10}Fc^+|Me_{10}Fc$ (decamethylferricinium|decamethylferrocene) reference redox couple, currently proposed as an improved alternative [30–32] to ferrocene, proposed by IUPAC [33,34], and whose formal redox potentials in water are −0.126 V and −0.283 V against our aqueous SCE reference and against ferrocene, respectively. The cell was thermostated at 298 K and the solutions were carefully deareated by nitrogen bubbling before the scans. The optimized polishing procedure for the working GC electrode consisted of surface treatment with diamond powder (Aldrich, diameter 1 μm) on a wet cloth (DP-Nap, Struers).

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