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A new ferrocene conjugate of a tyrosine PNA monomer: synthesis and electrochemical properties

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Dedicated to Dr. Richard Fish on the occasion of his 65th birthday.

Abstract

A new chiral ferrocene-labelled tyrosine PNA monomer **1** has been synthesised in good yield in both racemic and enantiomerically pure forms. It is suitable for insertion in various positions of PNA oligomers, a possibility that has been preliminarily demonstrated by synthesising the dimer **16.** Moreover, in view of possible applications to nucleic acid detection, a preliminary voltammetric investigation on the electrochemical activity of monomer **1** and its synthetic precursors has been carried out in DMF. It appears that, despite the bulkiness of the PNA monomer backbone, its insertion on the ferrocene group only moderately lowers the latter's diffusion coefficients and peak currents, thus affording voltammetric detection limits in the order of 10^{-6}–10^{-7} M.

Keywords: Ferrocene; Mitsunobu; Tyrosine PNA monomer; Bioorganometallic chemistry

1. Introduction

Peptide nucleic acids (PNAs) represent a very promising class of artificial DNA analogues with excellent DNA and RNA binding properties [1]. The stability of PNA/DNA complexes makes PNAs powerful tools in anti-sense and anti-gene applications, and as markers in DNA screening assays. In particular, the development of DNA biosensors based on PNA recognition layers is a new and exciting area in analytical chemistry because of the higher specificity, greater discrimination for single-base mismatches and faster hybridisation of PNAs [2]. However, in view of large-scale applications, improving PNA biosensor sensitivity is already a valuable target. This could be done by conjugating PNA to an organometallic moiety as a number of metal complexes have intense and easily identifiable spectroscopic or electrochemical signals that are different from those of the organic bulk of biomolecules. Many examples of the usefulness of metal-complexes in labelling biomolecules have so far been reported [3], among which the extensive and original work of Jaouen concerning the use of metal carbonyl complexes as molecular probes detectable by IR spectroscopy deserves particular mention [4].

We have recently reported the synthesis of PNA monomers labelled with arene $Cr(CO)_3$ [5] or Fischer-type carbene complexes [6] which are easily detected by FT-IR spectroscopy even at very low concentrations (10^{-7} M).

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Another suitable candidate marker for PNAs is the ferrocene group, which undergoes a stable reversible redox reaction and can be detected as such, or by adding a bioelectro-catalytic amplification step pivoting on the glucose-oxidase mediated oxidation of glucose [7]. Ferrocene has been widely used as an active probe for DNA detection [8], but only a few examples of ferrocene-labelled PNA have been reported: the reaction of ferrocenecarboxylic acid with the terminal amino group of a PNA heptamer [9], and the synthesis of a PNA oligomer in which a ferrocene unit was attached to a nucleobase [10].

The aim of our study was to synthesise ferrocenyl multi-labelled PNA oligomers, an objective that can be reached by inserting a number of mono-labelled monomers along the chain or by introducing a single molecule containing multiple ferrocenes.

We first considered the synthesis of a suitable mono-labelled monomer. We here describe the preparation of the chiral ferrocenyl-labelled tyrosine PNA monomer **1** in racemic and enantiomerically pure forms, in which the organometallic moiety is bound to the phenolic group of the tyrosine residue (Fig. 1). In addition to its chirality [11], monomer **1** meets two important requisites: it can be inserted in various oligomer positions (using either amino or carboxy functions) and the "label" does not modify any of the hydrogen-bond sites of the nucleobases.

2. Results and discussion

The synthesis of a tyrosine PNA monomer has previously been reported [12], and we considered the Mitsonobu reaction with ferrocenemethanol **5** a suitable method for labelling the tyrosine OH group. Looking at the scheme for obtaining monomer **4** (Scheme 1), in principle, the ferrocene unit could be introduced on the starting amino acid **2**, on the backbone **3**, or directly on **4**.

We investigated all three protocols. We first tried the Mitsunobu reaction between the racemic tyrosine PNA monomer **4** and ferrocenemethanol **5** in THF at room temperature (Scheme 2), but the corresponding ferrocenyl conjugate **1** was obtained in low yield (28%) after a very long reaction time (72 h).

In the second route, (Scheme 4, path a), the Mitsunobu reaction was run on the *N*-Cbz-protected tyrosine methyl ester **6** under the same conditions, and gave the corresponding labelled amino acid **7** in good yield (81%) and in a relatively short reaction time (18 h). Removal of the Cbz group with ammonium formate and Pd/C afforded ferrocenyl-tyrosine methylester **8** (75%), which underwent reductive amination with *N*-Cbz-2-amino acetaldehyde **9,** using $NaCNBH_3$ and $ZnCl_2$, to give the labelled backbone **10** in 76% yield. Alternatively, compound **10** can be obtained in comparable yield (Scheme 3, path b) by running the Mitsunobu reaction on the backbone **3,** which was easily obtained by the reductive amination of tyrosine methyl ester **2** with **9**. The target PNA monomer **1** was eventually obtained in 70% yield after coupling **10** with carboxymethyl thymine **11** in DMF in the presence of DCC at room temperature.

All of the synthetic steps were performed on racemic tyrosine and both enantiomers. The enantiomeric purity of L and D monomer **1** was checked by HPLC equipped with a chiral OD column and was complete in both cases.

As mentioned above, the next step in this work should be the preparation of a multi-labelled PNA oligomer by inserting a number of ferrocene-monomers within the chain. As PNA oligomer synthesis is usually performed on solid phase [1], and requires selective hydrolysis of the ester group and the cleavage of *N*-Cbz protection, we first investigated the stability and reactivity of monomer **1** to these synthetic transformations. Ester hydrolysis was successfully performed using $Ba(OH)_2$ or LiOH at room temperature and, after acidification with a 1 M solution of $KHSO_4$, the corresponding acid **12** was isolated in respectively 95% and 80% yield (Scheme 4). The enantiomeric excess of **12** determined by HPLC was 88% with $Ba(OH)_2$ hydrolysis and 85% with LiOH hydrolysis.

The carbobenzyloxy group in monomer **1** was cleaved using ammonium formate and Pd/C or H_2, Pd/C and

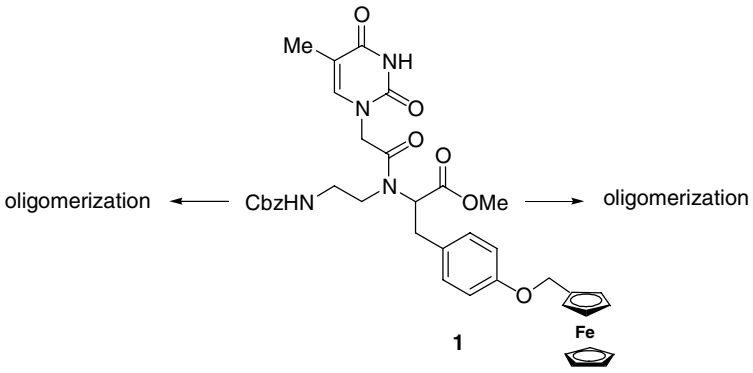

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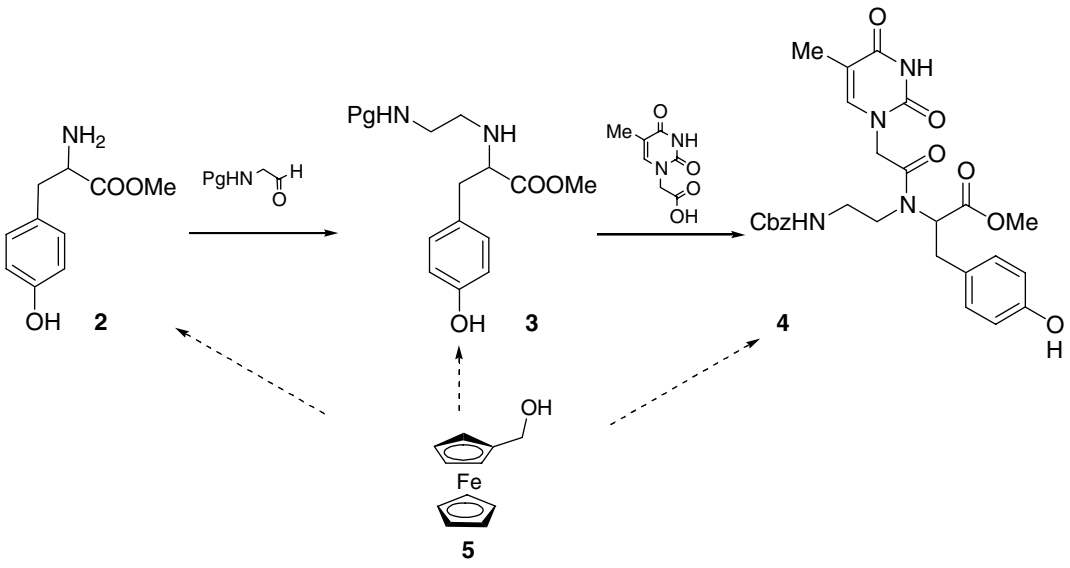
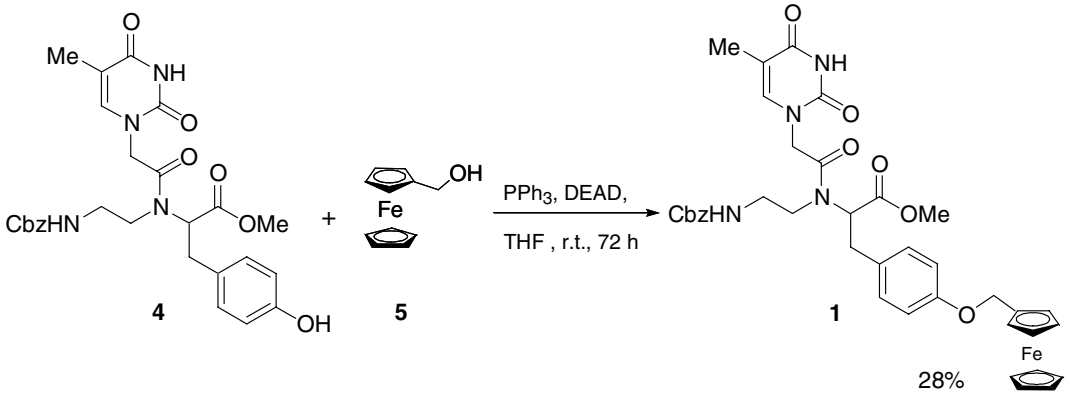
Fig. 1.



Scheme 1.



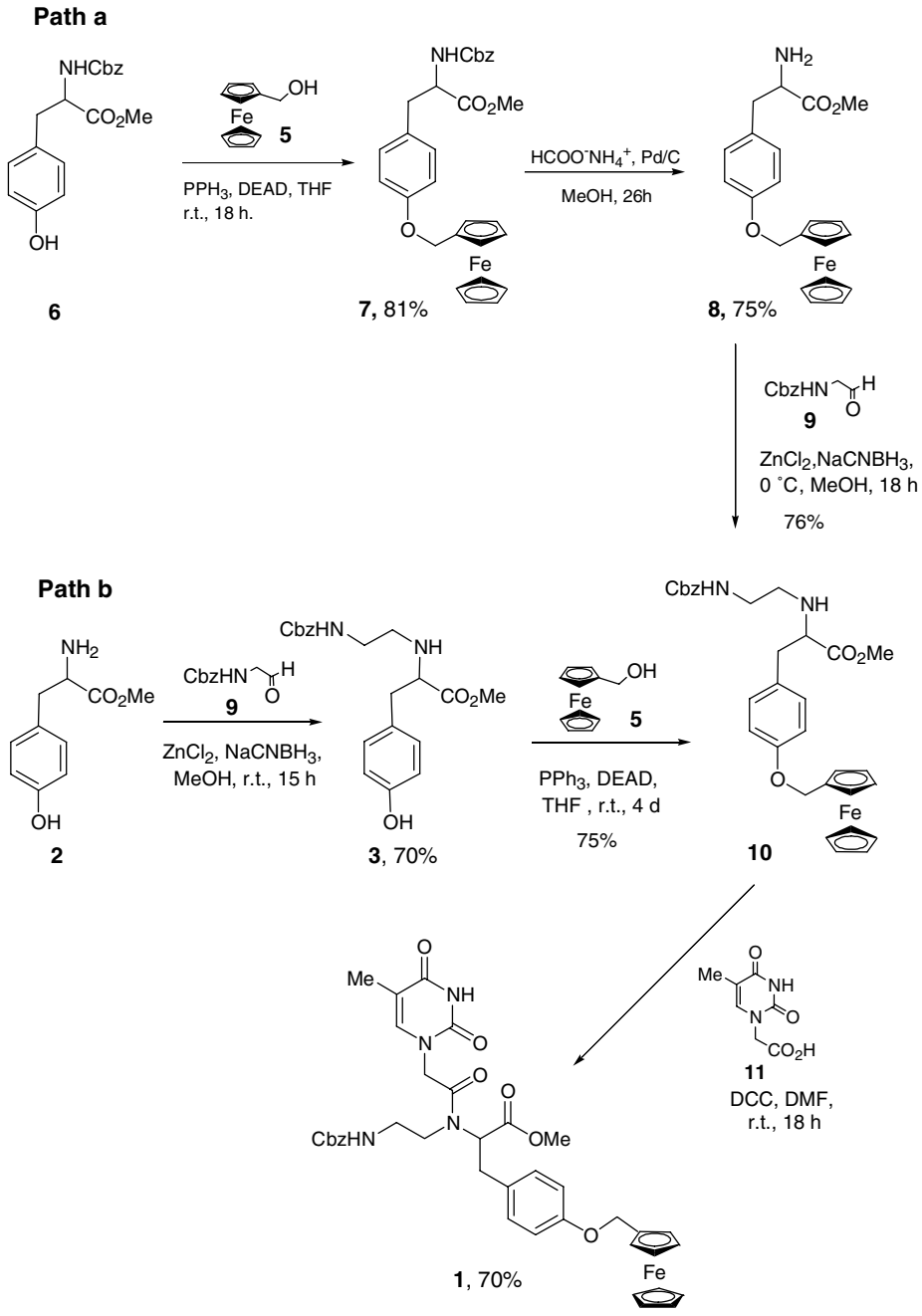
Scheme 2.

AcOH in methanol at room temperature; this did not produce any "free amino" monomer, but only gave ketopiperazine **13** as a result of the intramolecular attack of the deprotected amino group on ester carbonyl. To circumvent this problem we considered the synthesised dimer **15** in which intramolecular cyclisation could not take place. Racemic dimer **15** was obtained by reacting **12** and a classical glycine PNA monomer **14** [13] (Scheme 5). The condensation was run in DMF at room temperature using the trifluoroacetate of **14** in the presence of *N*,*N*-diisopropylethylamine (DIPEA) as base and diisopropylcarbodiimide (DIC) and 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (DhBtOH) as condensing agents. Compound **15** was isolated in good yield.

Dimer **15** was then hydrogenated with HCO_2NH_4 and Pd/C in MeOH at room temperature, and afforded the corresponding *N*-deprotected dimer **16** in 62% yield. This result is a good premise for the synthesis of ferrocene-multilabelled PNA oligomers.

3. Electrochemistry

In the perspective of using PNA oligomers as active DNA probes, it is important to evaluate how the functionalisation of ferrocene with the PNA moiety affects its electrochemical activity in terms of both redox potentials and currents. We therefore performed a preliminary voltammetric investigation of monomer **1**, synthetic intermediates **7**, **8** and **10**, and ferrocenemethanol **5** as a reference compound, in DMF + 0.1 M tetraethyl ammonium perchlorate (TEAP), using cyclic voltammetry (CV) on stationary Pt and glassy carbon (GC) electrodes (radius = 1 mm) at scan rates of 20–500 $mV\,s^{-1}$, and slow-scan voltammetry on Pt and GC rotating disk

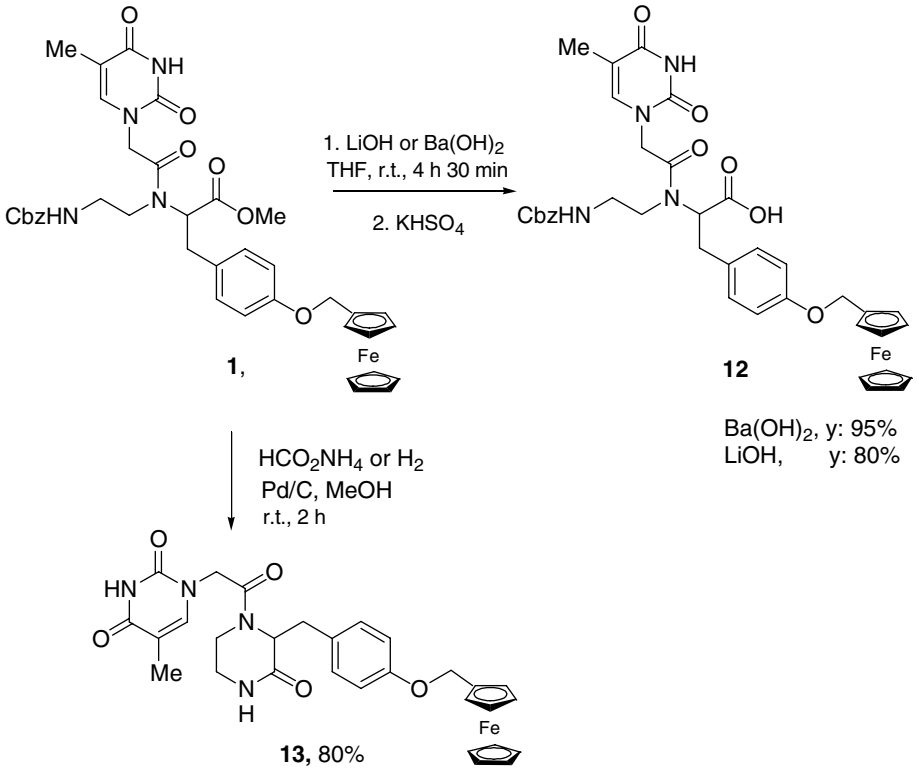


Scheme 3.

electrodes (RDE; radius = 1 mm) at a scan rate of 5 $mV\,s^{-1}$, in order to obtain a sound estimate of the diffusion coefficients from the limiting currents, according to Levich's equation [14].

The CV characteristics of the tested compounds in the case of the 0.02 $V\,s^{-1}$ scan rate are shown in Fig. 2 and the relevant features are summarized in Table 1. All of the compounds exhibit a single, reversible monoelectronic wave that is shifted in the positive direction for all ferrocenyl tyrosine derivatives with respect to ferrocenemethanol **5**. The observed potential shift remains almost unchanged with increasing complexity of the tyrosine compounds.

On the contrary, the currents appear to decrease with the increasing bulkiness of the ferrocene-labelled molecules, in accordance with the decrease in the relevant diffusion coefficients (Fig. 3) obtained by analysing the limiting current densities on RDE. However, this decrease is moderate (conveniently for our applicative aim) and the diffusion coefficients remain satisfactorily high (unlike, for example, in the case of a recent work by Kraatz et al. [15] in which



Scheme 4.

a low D range ($1–4 \times 10^{-7}$ $cm^2 s^{-1}$) was obtained for some ferrocene-monosaccharide compounds as a consequence of the high number of groups engaged in hydrogen bonding with the working aqueous medium).

Accordingly, preliminary investigation of the voltammetric detection limit for our monomer **1** showed that currents are still perceivable in the 10^{-6}–10^{-7} M range. The best results (Fig. 4) were obtained working in acetonitrile (which is less viscous than DMF, and thus enhances diffusion coefficients and peak currents), and changing simple CV for differential pulse voltammetry DPV [16].

4. Conclusions

A new chiral ferrocene-labelled PNA monomer **15** can be synthesised in good yield and complete enantiomeric excess when starting from L or D tyrosine. This monomer is suitable for insertion in various positions of PNA oligomers, as has been preliminarily demonstrated by synthesising dimer **16**. In addition in view of the applications of such labelled monomers to PNA biosensors, a preliminary voltammetric investigation on the electrochemical activity of monomer **1** has been carried out. It appears that, despite the bulkiness of the PNA monomer backbone, its insertion on the ferrocene group only moderately lowers the latter's diffusion coefficients and peak currents, thus affording voltammetric detection limits in the order of 10^{-6}–10^{-7} M.

The study of PNA monomers labelled with polyferrocene units [17] is currently in progress with the aim of improving sensitivity in ferrocene-based electrochemical biosensors.

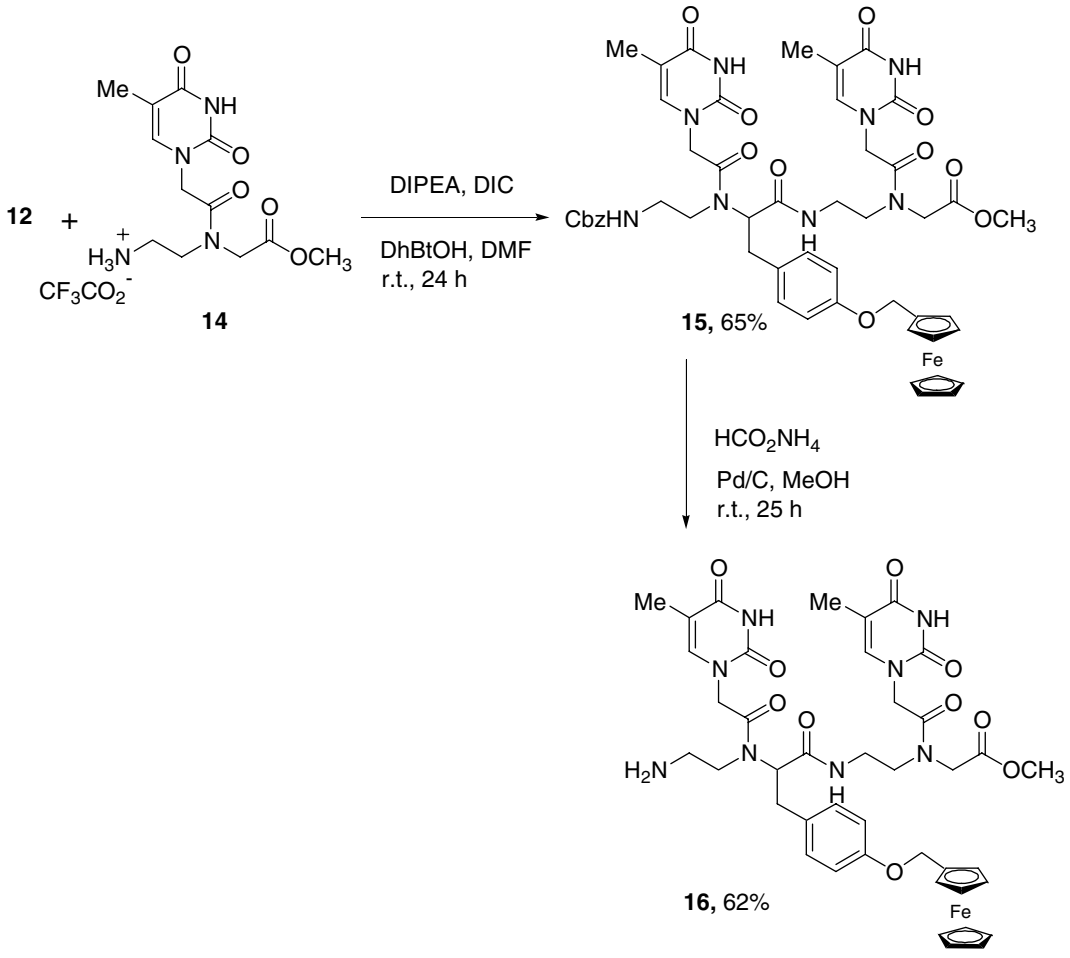
5. Experimental

5.1. General data

All reactions were performed under nitrogen atmosphere using a standard vacuum line. THF was distilled over sodium benzophenone ketyl, other reagent grade solvent were dried by standard procedures. Column chromatography was performed using Merck silica gel 60 (70–230 mesh); ^{1}H NMR spectra were recorded on a Bruker AC 300 and AMX 300 MHz. IR spectra were recorded on a Perkin–Elmer 1725X FT-IR. HPLC analyses were run on Agilent 1100 series, using a chiralcel OD column. Melting points were measured with a Büchi B-540 apparatus and are uncorrected. Optical rotations were measured using a Perkin–Elmer 243 polarimeter.

5.2. Ferrocenemethanol (*5*)

Prepared according literature report [18]. m.p. 78–81 °C (pentane). ^{1}H NMR ($CDCl_3$, δ): 1.5 (bs, 1H, OH);



Scheme 5.

4.16–4.17 (m, 5H); 4.22–4.24 (m, 2H); 4.71 (d, 2H, $J = 6.1$ Hz).

5.3. *N-(2-Cbz-aminoethyl)tyrosine methyl ester (**3**)*

A mixture of $ZnCl_2$ (4.2 mmol, 0.6 eq) and $NaCNBH_3$(8.4 mmol, 1.2 eq) in dry MeOH (7 ml) was slowly dropped at 0 °C into a solution of *N*-Cbz-2-aminoacetaldehyde **9** (7.0 mmol, 1 eq) and tyrosine methyl ester **2** (7.7 mmol, 1.1 eq.) in MeOH (8 ml). The mixture was stirred for 18 h at room temperature. After evaporation of the solvent, the residue was taken up with CH_2Cl_2 (50 ml) and washed with 50 ml of $NaHCO_3$ saturated solution, the aqueous phase was then extracted with CH_2Cl_2 (3×50 ml). The combined organic phases were washed with water, dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography on silica gel (AcOEt; $R_f = 0.40$) affording compound **3** in 77% yield.

(D,L)-**3** white powder, m.p. 87–88 °C (pentane). Anal. Calc. for $C_{20}H_{24}N_2O_5$: C, 64.50.; H, 6.50; N, 7.52. Found: C, 63.96; H, 6.52; N, 7.49%.

(L)-**3**, pale yellow oil. $[\alpha]_D^{20} = +4.27$ ($c = 4.3$ in $CHCl_3$); (D)-**3**, $[\alpha]_D^{20} = -4.98$ (c = 4.8 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, δ): 2.5–2.6 (m, 1H, CH_2NH); 2.7–2.9 (m, 3H, CH_2NH + CH_2PhOH); 3.1–3.2 (m, 2H, CH_2NHCbz); 3.4–3.5 (m, 1H, $CHCO_2Me$); 3.65 (s, 3H, OCH_3); 5.0 (bs, 2H, $PhCH_2O$); 5.1 (bs, 1H, OH); 5.3 (bs, 1H, OCONH); 6.7 (d, 2H, PhOH; $J = 8.4$ Hz); 7.0 (d, 2H, PhOH; $J = 8.4$ Hz); 7.3–7.4 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, δ): 38.5, 40.5, 47.12, 51.9, 62.5, 66.7, 115.5, 127.9, 128.3, 128.5, 130.0, 155.0, 156.7, 174.9. MS, (EI) *m/z* 372 (M^+). IR (neat, ν cm^{-1}): 3354 (OH), 1698 (CO).

5.4. *Synthesis of tyrosine PNA monomer (**4**)*

A mixture of DCC (2.6 mmol), carboxymethyl thymine **11** (2.6 mmol) and DhBtOH (2.6 mmol) in dry DMF (7 ml) was stirred for 1 h at room temperature. From the yellow–green solution a precipitate of DCU was formed. A solution of **3** (1.6 mmol) in dry DMF (4 ml) was added to the suspension and the reaction was stirred for 18 h at room temperature. The DCU was filtered off and, after distillation of the solvent, the residue was taken up with AcOEt (40 ml) and washed

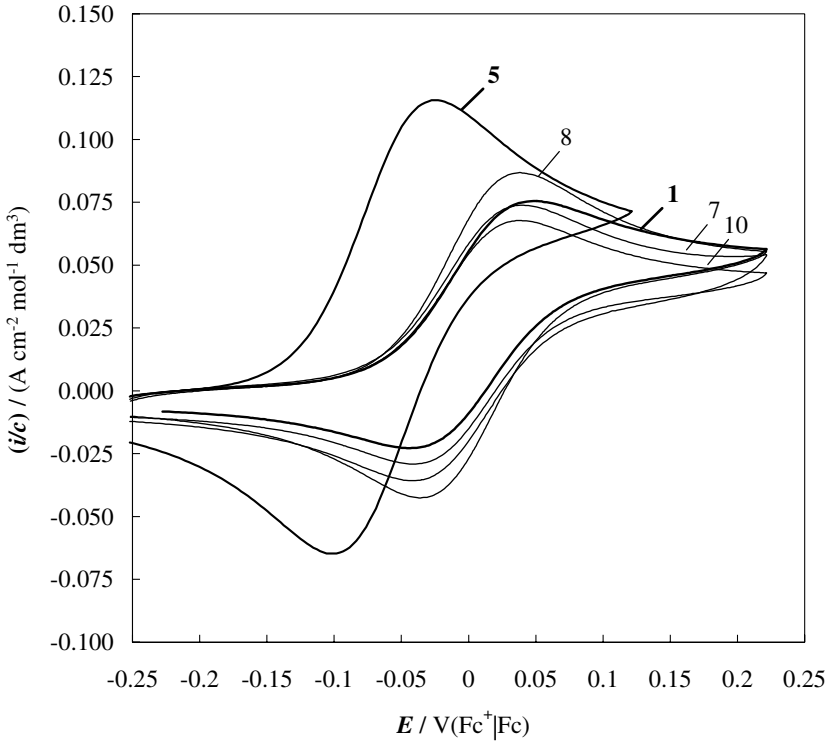


Fig. 2. A synopsis of the CV characteristics of the tested substrates, obtained on the GC electrode, in DMF + 0.1 M TEAP medium, at a 20 $mV\,s^{-1}$ scan rate and at 298 K.

Table 1
Selected CV features obtained for the tested molecules on GC stationary disk electrodes (1 mm radius) in DMF + 0.1 M TEAP medium, at potential scan rate $v = 0.02\ V\,s^{-1}$, at 298 K: anodic peak potentials $E_{p,a}$; anodic half-peak widths $(E_p - E_{p/2})_a$; distance between anodic and cathodic peaks, $(E_{p,a} - E_{p,c})$; half-wave potentials $E_{1/2}$, calculated (1) as the average between anodic and cathodic peak potentials, or (2) from convolutive analysis of the CV characteristics; anodic peak current densities, normalized vs. the substrate concentration, $(i_{p,a}/c)$; and anodic to cathodic peak current ratios, $I_{p,a}/I_{p,c}$

Molecule	MW ($g\,mol^{-1}$)	c (mol dm^{-3})	$E_{p,a}$ ($V(Fc^+\|Fc)$)	$(E_p - E_{p/2})_a$ (V)	$(E_{p,a} - E_{p,c})$ (V)	$E_{1/2}$ (1) ($V(Fc^+\|Fc)$)	$E_{1/2}$ (2) ($V(Fc^+\|Fc)$)	$(i_{p,a}/c)$ ($A\,cm^{-2}\,mol^{-1}\,dm^3$)	$I_{p,a}/I_{p,c}$
1	736.59	0.000751	0.043	0.061	0.078	0.004	0.008	0.063	1.3
5	216.06	0.000756	−0.027	0.057	0.069	−0.062	−0.059	0.102	1.1
7	527.39	0.000746	0.036	0.058	0.076	−0.002	0.001	0.062	1.3
8	393.26	0.000780	0.037	0.059	0.069	0.003	0.003	0.079	1.2
10	570.46	0.000748	0.035	0.059	0.070	0	−0.001	0.057	0.8

All potentials are referred to the ferrocene couple in DMF solvent.

with a saturated solution of $NaHCO_3$ (40 ml). The aqueous phase was extracted with AcOEt (3×40 ml). The combined organic phases were washed with water (40 ml), dried over Na_2SO_4 and evaporated in vacuo. The crude product was purified by column chromatography on silica gel (AcOEt; $R_f = 0.28$) affording the PNA monomer **4** as white powder in 70% yield.

(D, L)-**4**: white powder, m.p. 181–182 °C (Et_2O); Anal. Calc. for $C_{27}H_{30}N_4O_8$: C, 60.22; H, 5.61; N, 10.40. Found: C, 58.96; H, 6.26; N, 10.73%.

(L)-**4**: white powder, m.p. 104–107 °C (pentane); $[\alpha]_D^{20} = -92.95$ (c = 0.47 in $CHCl_3$); (D)-4: $[\alpha]_D^{20} = +91.83$ (c = 0.4 in $CHCl_3$); ^1H NMR ($CDCl_3$, δ): 1.78 (s, 3H, CH_3Thym); 2.6 (bd, 1H, CH_2N, $J_{gem} = 15.5$ Hz); 3.0–3.03 (m, 1H, NHCH_2); 3.14–317 (m, 1H, CH_2PhOH); 3.25–3.27 (m, 1H, NHCH_2); 3.27–3.29 (m, 1H, CH_2PhOH); 3.45–3.47 (m, 1H, CH_2N, $J_{gem} = 15.5$ Hz); 3.77 (s, 3H, OCH_3); 3.82 (d, 1H, CH_2Thym, $J_{gem} = 16.2$ Hz); 3.84–3.86 (m, 1H, CH); 4.9 (d, 1H, CH_2Thym, $J_{gem} = 16.2$ Hz); 5.0 (d, 1H, $PhCH_2O$, $J_{gem} = 12.3$ Hz); 5.18 (d, 1H, $PhCH_2O$, $J_{gem} = 12.3$ Hz); 5.8 (bs, 1H, Cbz-N*H*); 6.7 (s, 1H, CH = Thym); 6.76 (d; 2H, PhOH, $J = 8.2$ Hz); 6.95 (d; 2H, PhOH, $J = 8.2$ Hz); 7.2–7.4 (m, 5H, Ph); 7.52 (bs, 1H, OH); 10.35 (s, 1H, NH Thym). ^{13}C NMR ($CDCl_3$, δ): 12.3, 33.11, 38.8, 48.7, 49.5, 52.83, 64.15, 66.9, 110.8, 115.5, 127, 128, 130.2, 136, 141, 151.6, 155.6, 156, 164.5 167, 170.9. MS (FAB^+) *m/z* 539 (M^+), 540 ($M^+ + 1$).

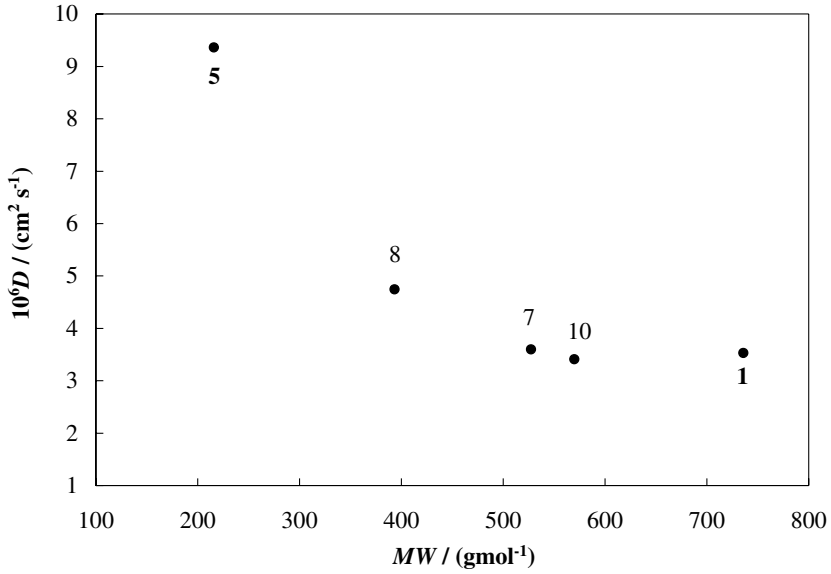


Fig. 3. Diffusion coefficients D of all tested substrates in DMF + 0.1 M TEAP at 298 K, obtained by RDE analysis and plotted against molecular weights MW.

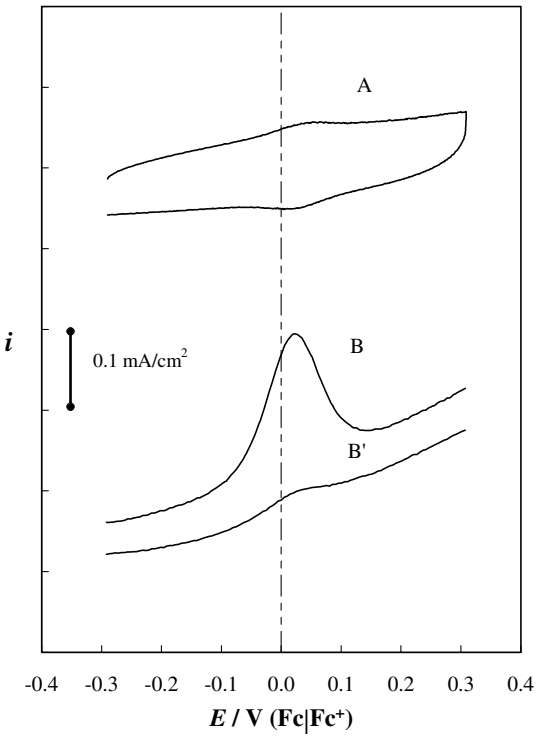


Fig. 4. Voltammetric characteristics obtained on GC disk electrodes (radius = 1.5 mm), at 298 K, working with low-concentration solutions of PNA monomer **1** in ACN + 0.1 M TBAP: A: CV (0.2 Vs^{-1}), 3.7×10^{-6} M substrate; B: DPV (0.05 Vs^{-1}) 3.7×10^{-6} M substrate; B′: DPV (0.05 Vs^{-1}), 3.7×10^{-7} M substrate.

5.5. O-Ferrocenyl-N-Cbz-tyrosine methyl ester (7)

N-Cbz-tyrosine methyl ester **6** (0.96 mmol, 1 eq) and PPh_3 (0.96 mmol, 1 eq) were added at room temperature to a solution of ferrocenemethanol (0.7 mmol, 0.73 eq) in dry THF (3 ml). The mixture was cooled at −30 °C and DEAD (0.96 mmol, 1 eq, 0.48 ml of 2 M solution in THF) was slowly added. The orange solution was stirred at room temperature for 18 h. After evaporation of the solvent, the residue was dissolved in 30 ml of CH_2Cl_2 and washed with brine (30 ml). The aqueous phase was extracted with of CH_2Cl_2 (3×30 ml). The combined organic phases were dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography on silica gel (Light petroleum/AcOEt, 7:3; $R_f = 0.30$) affording **7** in 81% yield.

(D, L)-**7**: orange powder; m.p. 112–113 °C (pentane). Anal. Calc. for $C_{29}H_{29}FeNO_5$: C, 66.04; H, 5.54; N, 2.66. Found: C, 66.10; H, 5.56; N, 2.57%.

(L)-**7** orange oil; $[\alpha]_D^{20} = +31.95$ (c = 1.9 in $CHCl_3$); (D)-**7** orange oil; $[\alpha]_D^{20} = -32.47$ (c = 1.73 in $CHCl_3$).

^{1}H NMR ($CDCl_3$, δ): 3.1 (bs, 2H, CH_2PhOH); 3.7 (s, 3H, OCH_3); 4.1–4.2 (m, 7H, CH, Fc); 4.3 (s, 2H, CH, Fc); 4.63 (m, 1H, CH); 4.75 (s, 2H, $FcCH_2O$); 5.1 (s, 2H, $PhCH_2O$); 5.2 (bs, 1H, NH); 6.8 (d, 2H, Ph, $J = 8.6$ Hz); 7.1 (d, 2H, Ph, $J = 8.6$ Hz); 7.3–7.4 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$), δ: 37.12, 52.08, 54.8, 66.4, 66.7, 68.4, 69.0, 82.35, 114.7, 127.6, 127.9, 128.3, 130.0, 136, 153, 157.8, 171.9. MS (ESI) *m/z*: 527 (M^+), 527 ($M^+ + 1$). IR ($CHCl_3$, ν cm^{-1}): 1718 (NHCO).

5.6. O-Ferrocenyltyrosine methyl ester (8)

At room temperature 30 mg of Pd/C (10%) and HCO_2NH_4 (0.7 mmol, 2.3 eq.) were added to a solution of **7** (0.3 mmol., 1 eq) in 6 ml of a mixture of CH_2Cl_2/MeOH, 3:2. The reaction was stirred overnight at room temperature, then filtered over a pad of celite. The

solvent was evaporated, the residue was dissolved in AcOEt (10 ml) and washed with brine (2 × 10 ml), the aqueous phase was extracted with AcOEt (3 × 15 ml). The combined organic layers, after washing with H_2O (10 ml), were dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography on silica gel (AcOEt; $R_f = 0.2$) affording compound **8** in 75% yield.

(D, L)-**8**: orange powder, m.p. 88–90 °C (pentane). Anal. Calc. for $C_{21}H_{23}FeNO_3$ (393,26): C, 64.14; H, 5.90; N, 3.56. Found: C, 63.99; H, 5.92; N, 3.55%.

(D)-**8** orange powder; m.p. 107–108 °C (pentane); $[\alpha]_D^{20} = -12.5$ (c = 0.172 in $CHCl_3$); L-**8** $[\alpha]_D^{20} = +14.73$ (c = 0.296 in $CHCl_3$). ^{1}H NMR ($CDCl_3$, δ): 1.6 (bs, 2H, NH_2); 2.7–2.8 (dd, 1H, CH_2Ph, $J_{gem} = 13.6$ Hz, $J_{vic} = 5.1$ Hz); 3.0–3.1 (dd, 1H, CH_2Ph, $J_{gem} = 13.6$ Hz, $J_{vic} = 5.1$ Hz); 3.68–3.7 (m, 1H, CH); 3.71 (s, 3H, OCH_3); 4.17–4.2 (m, 7H, CH, Fc); 4.3–4.32 (t, 2H, CH, Fc, $J = 1.84$ Hz); 4.76 (s, 2H, OCH_2Fc); 6.87 (d, 2H, Ph, $J = 8.5$ Hz); 7.1 (d, 2H, Ph, $J = 8.5$ Hz). ^{13}C NMR ($CDCl_3$, δ): 40.1, 52.0, 55.9, 66.6, 68.6, 69.14, 82.3, 114.9, 129.1, 130.2, 157.8, 174.8. MS (EI) *m/z*: 393 (M^+). IR ($CHCl_3$, ν cm^{-1}): 3500 (NH_2), 1735 (CO).

5.7. Synthesis of *10* via reductive amination reaction between *8* and *9*

A mixture of $ZnCl_2$ (0.165 mmol) and $NaCNBH_3$ (0.33 mmol) in dry MeOH (1 ml) was slowly added at 0 °C into a solution of *N*-Cbz-2-aminoacetaldehyde (**9**) (0.29 mmol) and **8** (0.30 mmol) in dry MeOH (2 ml). The reaction was complete after stirring 18 h at room temperature. After evaporation of the solvent, the residue was taken up with CH_2Cl_2 (10 ml) and washed with a saturated solution of $NaHCO_3$(10 ml) and the aqueous phase was extracted with CH_2Cl_2 (3 × 10 ml). The combined organic phases were washed with water, dried over Na_2SO_4 and evaporated in vacuo. The crude product was purified by column chromatography on silica gel (Light petroleum/AcOEt = 3/7; $R_f = 0.32$) affording compound **10** as orange oil in 76% yield.

(D, L)-**10**: orange oil, (D)-**10**, orange oil, $[\alpha]_D^{20} = -6.09$ (c = 0.95 in $CHCl_3$). (L)-**10**: $[\alpha]_D^{20} = +5.62$ (c = 0.97 in $CHCl_3$);. ^{1}H NMR ($CDCl_3$, δ): 2.54–2.56 (m, 1H, CH_2NH); 2.75–2.76 (m, 1H, CH_2NH); 2.80–2.82 (m, 1H, CH_2PhO); 2.90–2.91 (m, 1H, CH_2PhO); 3.1–3.3 (m, 2H, NHCH_2); 3.43 (bt, 1H, CH); 3.66 (s, 3H, OCH_3); 4.18 (s, 5H, CH, Fc); 4.19 (t, 2H, CH, Fc, $J = 1.8$ Hz); 4.30 (t, 2H, CH, Fc, $J = 1.8$ Hz); 4.74 (s, 2H, OCH_2Fc); 5.1 (s, 2H, $PhCH_2$); 5.20 (bs, 1H, NHCbz); 6.86 (d; 2H, PhO, $J = 8.6$ Hz); 7.07 (d; 2H, PhO, $J = 8.6$ Hz); 7.33–7.35 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$, δ): 38.6, 40.5, 47.2, 51.7, 62.5, 66.5, 68.5, 69.1, 82.6, 114.4, 128.0, 128.4, 129, 130.0, 136.7, 157.8, 156.5, 174.8. HRMS calc. for $C_{31}H_{34}FeN_2O_5$: 570.1800. Found 570.1802. IR ($CHCl_3$, ν, cm^{-1}) 1698-1718 (CONH).

5.8. Synthesis of *10* via Mitsunobu reaction between *5* and *3*

At room temperature **3** (5.4 mmol, 1.2 eq) and PPh_3 (5.4 mmol, 1.2 eq) were added to a solution of ferrocenemethanol (4.5 mmol, 1 eq) in dry THF (24 ml). The mixture was cooled at 0 °C and DEAD (5.4 mmol, 1.2 eq., 2.7 ml of 2 M solution in THF) was slowly added. The orange solution was stirred at room temperature for four days. After evaporation of the solvent, the residue was dissolved in CH_2Cl_2 (150 ml) and washed with brine (150 ml). The aqueous phase was extracted with CH_2Cl_2 (3 × 150 ml). The combined organic phases were dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography on silica gel, as reported above, affording **10** in 75% yield.

5.9. Synthesis of ferrocene-labelled monomer *1* by coupling of *10* with carboxymethyl thymine *11*

A mixture of DCC (0.26 mmol), carboxymethyl thymine **11** (0.26 mmol) and DhBtOH (0.26 mmol) in dry DMF (1.5 ml) was stirred for 1 h at room temperature. From the yellow–green solution a precipitate of DCU was formed. A solution of **10** (0.16 mmol) in dry DMF (1.5 ml) was added to the suspension and the mixture stirred for 20 h at room temperature. The DCU was filtered off and, after distillation of the solvent, the residue was taken up with AcOEt (10 ml) and washed with a saturated solution of $NaHCO_3$ (10 ml). The aqueous phase was extracted with AcOEt (3 × 10 ml). The combined organic phases were washed with water (10 ml), dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography on silica gel with AcOEt/Light petroleum = 6/4 affording the PNA monomer **1** as orange powder in 70% yield.

(D, L)-**1**: Orange powder, m.p. 181–182 °C (pentane); Anal. Calc. for $C_{38}H_{40}N_4O_8Fe$: C, 61.96; H, 5.47; N, 7.58. Found: C, 60.98; H, 5.50; N, 7.48%.

(L)-**1**: orange powder, 145–150 °C (pentane), $[\alpha]_D^{20} = -93.3$ (c = 1.0 in $CHCl_3$, e.e. >99% (HPLC, chiral OD column, hexane/isopropanol:70/30, 0.6 ml flow); (D)-**1**: $[\alpha]_D^{20} = +90.7$ (c = 0.63 in $CHCl_3$), e.e. >99% (HPLC). ^{1}H NMR ($CDCl_3$, δ): 1.85 (s, 3H, CH_3 Thym); 2.6 (d, 1H, CH_2N, $J = 15$ Hz,); 3.0–3.4 (m, 5H, $NCH_2CH_2N + CH_2PhO$); 3.78 (bs, 4H, $OCH_3 + CH$); 3.93 (d, 1H, $J = 16.3$ Hz CH_2CO); 4.19 (bs, 7H, CH,Fc); 4.30 (bs, 2H, CH, Fc); 4.58 (d, 1H, $J = 16.3$ Hz, CH_2CO); 4.77 (s, 2H, CH_2Fc); 5.01 (d, 1H, $J = 12.2$ Hz, PhCH_2O); 5.19 (d, 1H, $J = 12.2$ Hz, PhCH_2O); 5.6 (bs, 1H, NHCbz); 6.61 (s, 1H, CH═); 6.88 (d, 2H,

PhO, $J = 8.4$ Hz); 7.05 (d, 2H, PhO, $J = 8.4$ Hz); 7.34–7.36 (m, 5H, Ph); 8.71 (s, 1H, NH Thym). ^{13}C NMR ($CDCl_3$, δ): 12.4, 33.20, 39.06, 48.44, 48.97, 52.80, 63.70, 66.5 66.6, 68.4, 68.9, 82.6, 110.4, 115.2, 128.5, 129.3, 130.1, 141, 141.1, 150.8, 155.6, 157.8, 164.1, 166.95, 170.8. MS, (FAB$^+$) *m/z*: 737 (M^+), 738 ($M^+ + 1$), 739 ($M^+ + 2$). IR (nujol, ν cm^{-1}) 1680, 1740 (CONH). UV (CH_2Cl_2): $C = 6.79 \times 10^{-5}$ M, $A = 0.997$, $\lambda_{max} = 266$ nm.

5.10. Synthesis of ferrocene-labelled monomer 1 "via" Mitsunobu reaction between monomer 4 and ferrocenemethanol

At room temperature PPh_3 (0.38 mmol, 1.4 eq) was added to a solution of ferrocenemethanol **5** (0.27 mmol, 1 eq) and monomer **4** (0.38 mmol, 1.4 eq) in dry THF (6 ml). The mixture was cooled at −30 °C and DEAD (0.38 mmol, 0.2 ml of 2 M solution in THF, 1.4 eq) was dropped into the solution, that was stirred for 72 h at room temperature. The solvent was evaporated and the residue taken-up with CH_2Cl_2 (30 ml) and washed with brine (30 ml), the aqueous phase was then extracted with CH_2Cl_2 (3×20 ml). The combined organic layers were dried over Na_2SO_4 and evaporated to give an orange residue that was purified by silica-gel column chromatography, (AcOEt). Monomer **1** was isolated in 28% yield.

5.11. Ester hydrolysis in monomer 10 to give 12 using $Ba(OH)_2$

At room temperature to a solution of monomer **1** (0.14 mmol, 1.0 eq) in CH_2Cl_2 (3 ml), a suspension of $Ba(OH)_2$ (0.21 mmol, 1.5 eq) in MeOH/H_2O,1:1 (3 ml) was added over 5 min. The reaction mixture was stirred at room temperature for 4 h and 30 minutes then was acidified to pH 1 with a solution of $KHSO_4$ 1 M. The layers were separated, the aqueous phase extracted with CH_2Cl_2(3×25 ml). The combined organic layers was washed with water (20 ml) dried over Na_2SO_4 and evaporated. The crude product was purifie by a column chromatography on silica gel (EtOAc/MeOH,9:1, $R_f = 0.1$) affording **12** in 90% yield.

(D, L)-**12**: Orange powder, m.p. 179–182 °C (pentane); (L)-**12**: orange powder, m.p.: 162–164 °C (pentane), $[\alpha]_D^{20} = -90.2$, 88%; e.e. (HPLC, chiral OD column, hexane/isopropanol: 50/50 + 0.1% TFA, 1.0 ml/min flow). ^{1}H NMR ($CDCl_3$, δ): 1.87 (s, 3H, CH_3,Thym); 2.5–3.9 (m, 8H, CH + CH_2); 4.19 (bs, 7H, Fc); 4.32 (bs, 2H, Fc); 4.60 (bs, 1H, CH_2CO); 4.78 (s, 2H, CH_2Fc); 5.29 (s, 2H, $PhCH_2O$); 5.58 (bs, 1H, NHCbz); 6.89 (bd, 3H, PhO $J = 8.4$ Hz + CH═); 7.04 (d, 2H, PhO, $J = 8.4$ Hz); 7.29 (m, 5H, Ph); 10.50 (s, 1H, COOH). ^{13}C NMR ($CDCl_3$, δ): 12.26, 33.00, 38.52, 48.86, 49.17, 63.67, 66.60, 66.84, 68.74, 69.29, 82.61, 111.5, 115.03, 128.0, 128.38, 129.91, 130.1, 136.4, 141.03, 152.19, 156.68, 157.7, 164.04, 166.50, 172.94 (COOH). HRMS (EI): Calc. for $C_{37}H_{38}FeN_4O_8$. 722.20. Found: 722.2014. IR (nujol, ν, cm^{-1}): 1673 (CONH).

5.12. Synthesis of dimer 15

At room temperature **12** (0.54 mmol 1.1eq) was dissolved in dry DMF (5 ml). The yellow solution was cooled to −5 °C then DIC (0.97 mmol, 2.0 eq) and DhBtOH (0.97 mmol, 2.0 eq) were added. After stirring for 15 minutes, PNA monomer **14** [13] (0.49 mmol, 1.0 eq) dissolved in dry DMF (3 ml) was slowly added over 10 minutes while keeping the temperature around 0 °C, then DIEA(0.78 mmol;1.6 eq) was added and the mixture warmed to room temperature and stirred for 24 h. After distillation of DMF the thick liquid was dissolved in AcOEt (50 ml) and washed with a saturated solution of $NaHCO_3$(15 ml) and H_2O (15 ml). The organic phase was dried over Na_2SO_4, filtered and evaporated under vacuum. The crude product was purified by column chromatography on silica gel (eluent: EtOAc/MeOH 9:1) affording the *Z*-protected dimer **15** as yellowish white powder in 65% yield.

(D, L)-**15**, m.p.: 181–190 °C (pentane) Anal. Calc. For $C_{49}H_{53}FeN_8O_{12}$: C, 58.7; H, 5.30; N, 11.20; found: C, 57.37; H, 5.35; N, 11.36. ^{1}H NMR ($CDCl_3$, δ): 1.72, (s, 3H, CH_3 Thym); 1.81 (s, 3H, CH_3 Thym); 2.80–3.41 (m, 13H, CH + CH_2); 3.6 (s, 3H, OCH_3); 3.75 (s, 4H, CH_2 Thym); 4.2 (bs, 7H, CH, Fc); 4.31 (bs, 2H, CH, Fc); 4.7 (s, 2H, CH_2Fc); 5.0 (s, 2H $PhCH_2O$); 6.1 (bs, 1H NHCbz); 6.6–6.7 (s, 2H, CH═); 6.8 (d, 2H, PhO); 7.0 (d, 2H, PhO); 7.3–7.6 (m, 5H, Ph); 9.9 (s, 1H, NH Thym); 10.2 (s, 1H, NH Thym). ^{13}C NMR ($CDCl_3$, δ): 12.04, 12.26, 37.29, 39.27, 39.3, 47.99, 48.13, 48.6, 48.81, 52.64, 62; 66.56, 66.76, 68.75, 69.35, 82, 110.64, 110.21, 115.10, 128.01, 128.4, 130.14, 136.41, 141.31, 141.59, 151.18, 151.36, 157.8, 156.64, 164.70, 167.80, 168.59, 170.39, 171.15. HRMS Calc. for $C_{49}H_{53}FeN_8O_{12}$: 1001.30, Found 1002.32.

5.13. N-Cbz cleavage on 15 to give 16

At room temperature, $HCOONH_4$ (1.47 mmol, 10 eq) and 10% Pd/C (60 mg) were added to a solution of dimer 15 (0.147 mmol, 1.0 eq) dissolved in MeOH (3.5 ml). The reaction mixture was stirred for 25 h, then filtered through a celite pad and evaporated under vacuum. The crude product was purified by a column chromatography on silica gel (CH_2Cl_2/MeOH, 1:1; $R_f = 0.09$) affording **16** as yellowish white powder in 62% yield.

(L)-**15**: M.p: 212–215 °C $[\alpha]_D^{20} = -34.3°$ ($c = 0.172$ in MeOH); ^{1}H NMR ($CDCl_3$, δ): 1.83 (s, 3H, CH_3 Thym);

1.88 (s, 3H, CH_3 Thym); 2.5–3.4 (m, 13H, CH + CH_2); 3.71 (s, 3H, OCH_3); 3.76 (bs, 4H, CH_2 Thym); 4.19 (bs, 7H, CH, Fc); 4.3 (bs, 2H, CH, Fc); 4.7 (s, 2H, CH_2Fc); 6.8 (bs, 3H, PhO + CH=); 7.1 (d, 2H, PhO). ^{13}C NMR ($CDCl_3$, δ): 11.6, 11.8, 37.22, 39.1, 39.29, 47.2, 47.8, 48.14, 48.4, 48.7, 52.63, 60.57, 66.56, 68.5, 69.0, 82.0, 110.5, 110.2, 115.0, 127.8, 130.3, 141.3, 141.6, 151.3, 151.5, 156.0, 164.80, 167.9, 168.3, 169.7, 170.72 MS, ESI, 869.3 (M^+ + 2). IR (nujol, v, cm^{-1}): 1674 (CONH).

5.14. N-Cbz cleavage in **1** to give **13**

At room temperature Pd/C (10%) 15 mg and HCO_2NH_4 (0.35 mmol, 2.6 eq) were added to a solution of **1** (0.14 mmol, 1 eq) in 12 ml of DMF/MeOH, 1:1. The reaction was stirred for 2 h, then filtered over a pad of celite and the solvent evaporated. The residue was dissolved in CH_2Cl_2 (15 ml) and washed with brine (10 ml), the organic phase was dried over Na_2SO_4 and evaporated. The crude product was taken up with CH_2Cl_2 (3 ml) and the yellow solid was filtered, washed with CH_2Cl_2 (3×2 ml) and dried (80%).

(D, L)-**13**: orange powder, m.p. 221–225 °C (pentane); ^{1}H NMR (DMSO, δ): two rotamers A and B are present, A > B: 1.77 (s, 3H, CH_3, Thym, A); 1.73 (s, 3H, CH_3 Thym, B) 2.6–3.6 (m, 9H, CH + CH_2, A + B); 4.5–4.7 (m, 9H, CH + CH_2, A + B); 4.19 (bs, 4H, CH, Fc, A + B); 4.22 (bs, 10H, CH, Fc, A + B); 4.33 (bs, 4H, Fc, A + B); 4.78 (s, 2H, CH_2Fc, A); 4.81 (s, 2H, CH_2Fc, B); 6.66 (s, 1H, CH=, B); 6.88 (d, 2H, PhO, J = 8.5 Hz, A); 6.97 (d, 2H, PhO, J = 8.5 Hz, B); 7.07 (d, 2H, PhO, J = 8.5 Hz, A); 7.23 (d, 2H, PhO, J = 8.5 Hz); 7.28 (s, 1H, CH=, A); 8.10 (s, 1H, NH, A); 8.14 (s, 1H, NH, B); 11.25 (s, 2H, NH Thym, A + B).^{13}C NMR (DMSO, δ): (two rotamers): 12.35, 35.30, 36.07, 36.50, 48.20, 48.65, 56.94, 59.1 66.45, 66.61 68.74, 68.9, 69.88, 82.4, 82.6, 108.5, 108.6, 114.8, 115.2 129.4, 129.5, 129.9, 131.3, 142.04, 142.61, 151.32, 151.5, 157.7, 158.0, 164.8, 164.9, 165.97, 166.06, 167.8, 168.3. MS (EI) *m/z* 570 (M^+); HRMS (ESI) for $C_{29}H_{30}FeN_4O_5$ found: 568.16060. IR (nujol, v cm^{-1}) 1660 (CONH).

6. Electrochemical studies

The experiments were performed on carefully deaerated solutions in a cell thermostated at 298 K, by an Autolab PGSTAT 12 potentiostat/galvanostat (EcoChemie, The Netherlands) run by a PC with GPES software and equipped with a Metrohm 663 VA Stand including a Pt or GC rotating disk working electrode, a carbon counter−electrode, and a saturated calomel electrode (SCE), inserted into a jacket containing 3 M aqueous KCl and ensuring contact with the working solution via a glass joint, in order to avoid precipitation of the tested substrates within the SCE porous frit.

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