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# Synthesis of the first chiral PNA monomer labelled with a Fischer-type carbene complex

Stefano Maiorana [a,*], Emanuela Licandro [a,*], Dario Perdicchia [a], Clara Baldoli [a], Barbara Vandoni [a], Clelia Giannini [a], Michele Salmain [b]

[a] *Dipartimento di Chimica Organica e Industriale, Centro di Eccellenza CISI, Università di Milano, and CNR-Istituto di Scienze e Tecnologie Molecolari, Via C. Golgi 19, I-20133 Milan, Italy*

[b] *Laboratoire de Chimie et Biochimie des Complexes Moléculaires, Ecole Nationale Supérieure de Chimie de Paris, 11, Rue Pierre et Marie Curie, F-75231 Paris, France*

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Dedicated to Professor Renato Ugo on the occasion of his 65th birthday

**Abstract**

The synthesis, through a cross-metathesis reaction, of the first chiral peptide nucleic acid (PNA) monomer labelled with a Fischer-type carbene complex of chromium is reported. IR analysis of the new bioconjugate shows that the $Cr(CO)_4$ moiety represents a suitable spectroscopic probe for diagnostic purposes.


*Keywords:* Peptide nucleic acids; Fischer carbene complexes; Cross-metathesis; Biorganometallics

## 1. Introduction

Bio-organometallic chemistry is a rapidly growing borderline area partially superimposing to organic, inorganic and medicinal chemistry. The opportunities offered a priori in such a field for searching of new therapeutic and diagnostic agents are really unique. The broad range of studies on the synthesis and applications of metal conjugates of biomolecules (sugars, aminoacids, nucleic acids, steroids, etc.) has been widely reviewed [1–4]. Recently, transition metal complexes of aminoacids and peptides have attracted even more attention because of their peculiar features due to the unique binding environments offered by the presence of the metal [5].

Peptide nucleic acids (PNA) **1** (Fig. 1) are DNA mimics in which the ribose-phosphate moiety is substituted with an *N*-(2-aminoethyl)glycine backbone, bearing the nucleobases attached to the glycine nitrogen atom through a $CH_2CO$-linker. Oligomers of such monomeric entities display high affinity for complementary DNA and RNA and therefore they are interesting for potential applications in diagnostic and as antisense or antigene drugs [6–8]. In contrast with the large number of studies using PNAs, few examples of metal-modified PNAs have been reported so far. Recently, Metzler-Nolte has published the synthesis, spectroscopic and electrochemical properties of PNA monomers of type **2** (Fig. 1) bearing different organometallic complexes on the terminal nitrogen of aminoethyl group [9,10].

* Corresponding authors. Tel.: +39-02-503-14142; fax: +39-02-503-14139.
*E-mail address:* stefano.maiorana@unimi.it (S. Maiorana).

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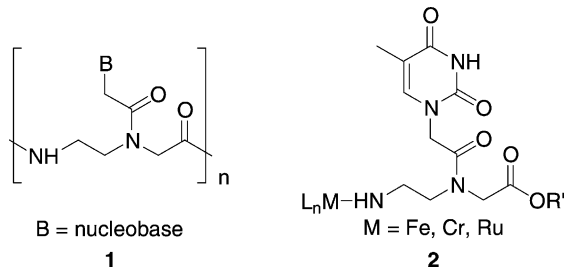



Fig. 1. PNA structure (**1**) and bioconjugate monomers (**2**).

We have recently addressed our research to the design of new organometallic conjugates of PNA monomers [11] (PNA monomers containing the $ArCr(CO)_3$ unit have been synthesised by us) trying to exploit our expertise in Fischer carbene and arene chromium carbonyl complexes chemistry [12,13]. In fact, such carbonyl complexes show peculiar and strong IR spectroscopic absorptions, due to the stretchings of the CO groups, in the region between 2070 and 1820 $cm^{-1}$ where no absorptions of organic molecules are usually present, thus providing a useful handle for the potential analytical detection of the metal-modified biomolecules. It is noteworthy, as we have already shown in collaboration with Jaouen group, that Fischer carbene complexes of chromium and tungsten can be successfully used to tag aminoacids and proteins and these organometallic conjugates are easily detected by IR spectroscopy even at very low concentration, with excellent sensitivity [14].

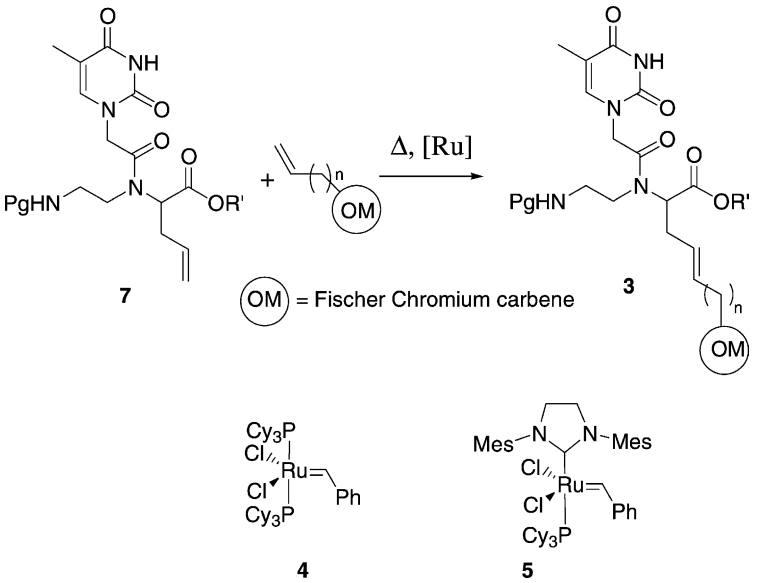
In this paper, we report the synthesis of chiral PNA monomers **3** in which the Fischer-type carbene functionality has been introduced through a cross-metathesis reaction using both the Grubbs catalysts **4** and **5** (Scheme 1).

As for some other nucleotide analogs, the chirality of PNA strand can affect the binding properties and therefore the interaction with the DNA [15,16]. Some examples of chiral PNA have been reported so far but no examples of chiral and metal-labelled PNA monomers are known. These organometallic conjugates are expected to show greater lipophilic character and a better cell permeability in comparison with standard PNAs. The metal-labelled allylglycine monomer **3** could be inserted into a classical PNA oligomer.

## 2. Experimental

### 2.1. Synthesis of *9a* and *9b*

2-Trimethylsilyl ethanol (0.70 ml, 4.88 mmol, 1.1 eq.) and a small amount of 4-dimethylaminopyridine (DMAP) were added under nitrogen to a suspension of




Scheme 1.

**8a** or **8b** (4.45 mmol, 1 eq.) in AcOEt (100 ml). Then at 0 °C, *N*,*N*′-dicyclohexylcarbodiimide (DCC) (0.916 g, 4.44 mmol, 1 eq.) was added portion wise. After stirring overnight at room temperature, DCU was filtered off, the filtrate was washed with water (3 × 20 ml) dried over $Na_2SO_4$ and the solvent evaporated. The crude product was purified by under vacuum silica gel column chromatography (eluent: AcOEt/hexane 1:9) affording **9a** or **9b**.

### 2.2. Product *9a*

Colourless oil. Yield: 96% $^1$H NMR ($CDCl_3$, 300 MHz), $\delta$ (ppm): 7.70–7.67 (m, 2H, arom.), 7.52–7.50 (m, 2H, arom.), 7.32–7.19 (m, 4H, arom.), 5.78–5.76 (m, 1H, C*H*=$CH_2$), 5.36 (d, $J = 8.16$ Hz, 1H, NH), 5.17–5.12 (m, 2H, CH=C*H*$_2$), 4.47–4.37 (m, 4H, C*H*$_2$OCO, COOC*H*$_2$CH$_2$Si), 4.27–4.24 (m, 2H, C*H*CH$_2$OCO, NHC*H*CO), 2.66–2.47 (m, 2H, CH$_2$=CHC*H*$_2$), 1.02 (t, $J = 8.6$ Hz, 2H, C*H*$_2$Si), 0.05 (s, 9H, Si(CH$_3$)$_3$). $^{13}$C NMR ($CDCl_3$, 75 MHz), $\delta$ (ppm): 171.6, 155.9, 144.2, 141.2., 133.6, 127.6., 127.0, 125.2, 119.9, 117.5, 66.3, 62.8, 53.9, 47.1, 35.9, 17.1, 14.1. FT-IR (neat), $\nu = 3342.1$ (NH), 1726.0 (CO) cm$^{-1}$. HR-MS: *m*/*z*: 437.1959.

### 2.3. Product *9b*

Colourless oil. Yield: 95%. $^1$H NMR ($CDCl_3$, 300 MHz), $\delta$ (ppm): 7.87–7.83 (m, 2H, arom.), 7.75–7.70 (m, 2H, arom.), 5.82–5.61 (m, 1H, C*H*=$CH_2$), 5.10–4.86 (m, 3H, CH=C*H*$_2$ + COC*H*N), 4.29–4.20 (t, $J = 3.3$ Hz, COOCH$_2$), 3.02–2.94 (t, $J = 11$ Hz, 2H, CH$_2$=CHC*H*$_2$), 1.01–0.92 (t, $J = 3.28$ Hz, 2H, COOCH$_2$C*H*$_2$), 0.08 (s, 9H, Si(CH$_3$)$_3$). $^{13}$C NMR ($CDCl_3$, 75 MHz), $\delta$ (ppm): 168.8, 167.4, 134.0, 133.3, 131.7, 130.8, 128.7, 123.6, 118.5, 64.3, 51.8, 33.2, 17.2, 15.9. FT-IR (neat): $\nu = 1777.9$ (CO Phth), 1713 (CO ester) cm$^{-1}$. HR-MS: *m*/*z*: 345.1400.

### 2.4. Synthesis of *10*

#### 2.4.1. Method a

Piperidine (4.4 ml) was added to a solution of **9a** (1.95 g, 4.45 mmol, 1 eq.) in dry $CH_2Cl_2$, under nitrogen and the mixture stirred for 90 min at room temperature. The solvent was evaporated and the product was purified by a silica gel column chromatography (eluent: AcOEt/light petroleum 6:4), affording 718 mg (3.32 mmol) of **10** (yield: 75%).

#### 2.4.2. Method b

Hydrazine monohydrate (3 eq., 1.84 ml) was added to a solution of **9b** (4.4 g, 1 eq.) in 94 ml di ethanol. After stirring at room temperature for 3 days, the ethanol and hydrazine were removed under vacuum. The residue was taken up with 20 ml of $H_2O$/AcOEt (1:1), the aqueous layer was extracted with AcOEt (3 × 15 ml) and the organic phase washed with $H_2O$ (20 ml), and brine (20 ml), dried on $Na_2SO_4$ and evaporated. The crude product was purified by a silica gel column chromatography (eluent: AcOEt), to afford 2 g of **10** (yield: 71.3%).

### 2.5. Product *10*

Colourless oil, $^1$H NMR ($CDCl_3$, 300 MHz), $\delta$ (ppm): 5.67–5.59 (m, 1H, C*H*=$CH_2$), 5.08–5.00 (m, 2H, CH=C*H*$_2$), 4.15 (t, $J = 8.3$ Hz, 2H, C*H*$_2$CH$_2$Si), 3.41 (t, $J = 6.21$ Hz, 1H, C*H*NH$_2$), 2.29–2.27 (m, 2H, CH$_2$= CHC*H*$_2$), 1.53 (bs, 2H, NH$_2$), 0.98 (t, $J = 8.3$ Hz, 2H, C*H*$_2$Si), 0.02 (s, 9H, Si(CH$_3$)$_3$). $^{13}$C NMR ($CDCl_3$, 75 MHz), $\delta$ (ppm): 175.1, 133.3, 118.5, 63.1, 53.7, 38.9, 17.3, 14.0. FT-IR (neat) 3380.6 (NH), 1733.7 (CO) cm$^{-1}$. MS (FAB$^+$): *m*/*z*: 215 $[M]^+$.

### 2.6. Synthesis of *11a* and *11b*

A solution of $NaCNBH_3$ (512 mg, 0.81 mmol, 1.2 eq.) and $ZnCl_2$ (11.4 mg, 0.08 mmol, 0.1 eq.) in 3 ml of dry MeOH at room temperature and under nitrogen atmosphere was added drop-wise to a stirred solution of **10** (160 mg, 0.74 mmol, 1.1 eq.) and fluorenylmethoxycarbonyl (Fmoc)–aminoacetaldehyde or Phth (phthalimido) acetaldehyde (0.68 mmol, 1 eq.) in 6 ml of dry MeOH. After stirring overnight at room temperature, the solvent was evaporated, and the residue taken up with AcOEt. The organic layer was washed with a saturated aqueous solution of $NaHCO_3$ and brine, then dried on $Na_2SO_4$. After evaporation of the solvent the residue was purified by a silica gel column chromatography, affording **11a** (67%) or **11b** (70%).

### 2.7. Product **11a**

Colourless oil. Yield: 67%. $^1$H NMR ($CDCl_3$, 300 MHz), $\delta$ (ppm): 7.78–7.74 (m, 2H, arom.), 7.51–7.62 (m, 2H, arom.), 7.41–7.30 (m, 4H, arom.), 5.87–5.66 (m, 1H, C*H*=$CH_2$), 5.36 (bs, 1H, NH–Fmoc), 5.16–5.77 (m, 2H, CH=C*H*$_2$), 4.40–4.06 (m, 5H, C*H*$_2$OCO, C*H*$_2$CH$_2$Si, C*H*CH$_2$OCO), 3.39–3.15 (m, 3H (t, $J$ = 6.2 Hz, Fmoc–NHC*H*$_2$) NHC*H*COO), 2.86–2.59 (m, 2H, OCONHC*H*$_2$), 2.40 (t, $J$ = 6.87 Hz, 2H, CH$_2$=CHC*H*$_2$), 1.65 (bs, 1H, CH$_2$N*H*CH), 1.01 (t, $J$ = 6.30 Hz, 2H, CH$_2$Si), 0.05 (s, 9H, Si(CH$_3$)$_3$). $^{13}$C NMR ($CDCl_3$, 75 MHz), $\delta$ (ppm): 174.6, 156.5, 144.5, 141.3, 133.6, 127.6, 127.0, 125.1, 119.9, 118.2, 66.7, 63.2, 60.6, 47.3, 47.1, 40.8, 37.6, 17.5, 14.2. FT-IR (neat), $\nu$ = 3338 (NH), 1728 (CO) cm$^{-1}$, MS (FAB$^+$): *m/z*: 481 $[M]^+$, 381 $[M + \text{H–CH}_2\text{CH}_2\text{Si(CH}_3)_3)]^+$.

### 2.8. Product **11b**

Yellow oil. Yield: 70%. $^1$H NMR ($CDCl_3$, 300 MHz), $\delta$ (ppm): 7.79–7.5 (m, 2H, arom.), 7.66–7.64 (m, 2H, arom.), 5.69–5.55 (m, 1H, C*H* = $CH_2$), 4.99–4.89 (m, 2H, C*H*=$CH_2$), 4.06 (t, $J$ = 8.3 Hz, 2H, COOCH$_2$), 3.72 (dt, $J$ = 2, 1 Hz, $J$ = 6 Hz, 2H, Phth–NC*H*$_2$), 3.27 (t, $J$ = 6.4 Hz, 1H, NHC*H*COO), 2.94–2.86 (m, 1H, C*H*–HNH), 2.75–2.67 (m, 1H, C*H*–HNH), 2.28 (t, $J$ = 6.7 Hz, 2H, CH$_2$CH=CH$_2$), 1.63 (bs, 1H, NH), 0.90 (t, $J$ = 8.3 Hz, 2H, C*H*$_2$Si(CH$_3$)$_3$), 0.25 (s, 9H, CH$_2$Si(CH$_3$)$_3$. $^{13}$C NMR ($CDCl_3$, 75 MHz), $\delta$ (ppm): 174.3, 168.3, 133.9, 133.5, 132.0, 123.2, 117.7, 62.8, 60.4, 45.8, 37.6, 17.4, 16.0. FT-IR 1774, 1714, MS (FAB$^+$): *m/z*: 389 $[M + \text{H}]^+$, 287 $[M - \text{CH}_2\text{CH}_2\text{Si(CH}_3)_3]^+$, 243 $[M - \text{COOCH}_2\text{CH}_2\text{Si(CH}_3)_3]^+$.

### 2.9. Synthesis of **7a** and **7b**

DCC (85 mg, 0.4 mmol, 2.0 eq.) was added to a stirred solution of 3-hydroxy-3,4-dihydro-4-oxo-1,2,3-benzotriazine (DHBT) (68.3 mg, 0.4 mmol, 2.0 eq.) and of thymine–1-acetic acid (77 mg, 0.4 mmol, 2.0 eq.) in 2 ml of dry DMF at 0 °C and stirred for 90 min. After 30 min at room temperature, DCU was filtered off and the filtrate added to a solution of **11a** or **11b** (0.208 mmol, 1 eq.) in 2 ml of dry DMF. After stirring overnight, the solvent was evaporated, the crude taken up with AcOEt, washed with saturated aqueous solution of $NaHCO_3$ and brine. The organic layer was dried on $Na_2SO_4$, the solvent evaporated, and the product purified by a silica gel column chromatography (eluent: light petroleum/AcOEt 1:1) affording **7a** (82%) or **7b** (80%).

### 2.10. Product **7a**

White solid. Yield: 82%. m.p. 78–81 °C (*n*-hexane). $^1$H NMR ($CDCl_3$, 300 MHz), $\delta$ (ppm): 8.68 (bs, 1H, NH thymine), 7.76–7.72 (m, 2H, arom.), 7.61–7.57 (m, 2H, arom.), 7.42–7.29 (m, 4H, arom.), 6.80 (s, 1H, =CH thymine), 5.95 (bs; 1H, Fmoc–NH), 5.76–5.65 (m, 1H, C*H*–CH$_2$), 5.50–5.20 (m, 2H, CH–C*H*$_2$), 4.56–4.32 (m, 2H C*H*CH$_2$OCO, COC*H*H–thymine), 4.3–3.69 (m, 6H, C*H*$_2$OCO, NHC*H*COO, C*H*$_2$CH$_2$Si, COC*H*H–thymine), 3.67–3.26 (m, 4H, OCONHC*H*$_2$C*H*$_2$), 2.77–2.75 (t, $J$ = 4.7 Hz, 2H, CH$_2$–CHC*H*$_2$), 1.85 (s, 3H, CH$_3$), 1.00 (t, $J$ = 8.58 Hz, 2H, CH$_2$Si), 0.22 (s, 9H, Si(CH$_3$)$_3$). $^{13}$C NMR ($CDCl_3$, 75 MHz), $\delta$ (ppm): 174.0, 166.3 , 164.0, 156.7, 151.2, 143.7, 141.2, 140.0, 133.0, 127.6, 127.0, 125.0, 119.9, 118.5, 110.3, 66.7, 60.8, 60.0, 56.9, 47.0, 39.7, 33.7, 32.8, 31.2, 17.3, 12.3. FT-IR (neat) $\nu$ = 3328, 3205 (NH), 1682 (CO) cm$^{-1}$, MS (FAB$^+$): *m/z*: 647 $[M + \text{H}]^+$, 574 $[M - \text{Si(CH}_3)_3]^+$, 545 $[M - \text{CH}_2\text{CH}_2\text{Si(CH}_3)_3]^+$, 481 $[M - \text{thymine–CH}_2\text{CO}]^+$, 380 $[M - \text{C}H_2\text{CH}_2\text{Si(CH3)–thymine–CH}_2\text{CO}]^+$. Anal. Calcd. for $C_{34}H_{42}N_4O_7Si$: C = 63.14%, H = 6.55%, N = 8.66%, found: C = 63.21%, H = 6.67%, N = 8.59%.

### 2.11. Product **7b**

White solid. Yield: 80%. m.p. 87–90 °C (*n*-hexane) $^1$H NMR ($CDCl_3$, 300 MHz), $\delta$ (ppm): 8.45 (bs, 1H, NH thymine), 7.87–7.81 (m, 2H, arom.), 7.76–7.68 (m, 2H, arom.), 7.01 (s, 1H, CH thymine), 5.82–5.68 (m, 1H, C*H*=CH$_2$), 5.23–5.07 (m, 2H, CH=C*H*$_2$), 4.75 (d, $J$ = 8.49 Hz, 1H, COCH–*H*–thymine), 4.48–4.37 (m, 1H, N*C*HCO), 4.20–3.98 (m (t, $J$ = 8.2 Hz), 5H, COOC*H*$_2$+COCH-*H*-thymine + Phth-NC*H*$_2$), 3.74–3.67 (m, 1H, thymine–NCH–*H*), 3.64–3.4 (m, 1H, thymine–NCH–*H*), 2.86–2.6 (m, 2H, C*H*$_2$CH=CH$_2$), 1.91 (s, 3H, CH$_3$ thymine), 0.98–0.91 (m, 2H, C*H*$_2$Si(CH$_3$)$_3$), 0.11 (s, 9H, Si(CH$_3$)$_3$). $^{13}$C NMR ($CDCl_3$, 75 MHz), $\delta$ (ppm): 170.0, 168.1,

167.9, 164.4, 150.9, 141.2, 134.3, 133.8, 132.0, 123.6, 119.8, 110.3, 63.9, 59.6, 48.4, 36.4, 32.9, 17.4, 12.2, 16.0. FT-IR. $\nu$ = 1774, 1714, 1680, 1627 $cm^{-1}$ MS (FAB$^+$): *m/z*: 555 ($M^+$ + H), 453 ($M^+$ + H–$CH_2CH_2Si(CH_3)_3$). Anal. Calcd. for $C_{27}H_{34}N_4O_7Si$: C = 58.47%, H = 6.18%, N = 10.10%, found C = 58.54%, H = 6.68%, N = 10.29%.

*2.11.1. General procedure for metathesis reactions*

To a solution of **7a** or **7b (**0.172 mmol, 1 eq.) in dry and degassed $CH_2Cl_2$ (1.5 ml), 100 mg (0.224 mmol, 1.3 eq.) of **6b** and 14 mg (0.017 mmol, 10 mol%) of **3** were added. The mixture was refluxed for 23 h. The solvent was evaporated and the residue was purified by silica-gel column chromatography (eluent: $CH_2Cl_2$/MeOH 9.6:0.4).

*2.12. Product **12a***

Orange oil. $^1$H NMR (300 MHz) $\delta$ (ppm): 8.22 (bs, 1H, NH thymine); 7.75–7.73 (m, 2H, arom.); 7.60–7.57 (m, 2H, arom.); 7.41–7.25 (m, 4H; arom.); 6.82 (s, 1H, CH=C thymine), 5.93 (bs, 1H, NH–Fmoc), 5.37–5.33 (m, 2H, C*H*=C*H*), 4.55–74.43 (m, 2H (d, $J$ = 6.90, COOCH–*H*), C*H*$CH_2$OCO), 4.31–4.16 (m, 6H, C$H_2$OCO, NHC*H*COO, COOCH–*H*–thymine, $SiCH_2CH_2$), 3.98–3.85 (m, 4H, C$H_2$ OC$H_2$), 3.70–3.34 (m, 6H, N–NC$H_2$, NC$H_2$C$H_2$ $COCH_2$–thymine, $CH_2NCH_{2ax}$), 2.98–2.60 (m, 9H, $CH_2NCH_{2eq}$, C$H_2$CH = CHC$H_2$, 2.83 (s, $CH_3$, *cis*), 2.81 ($CH_3$, s, *trans*)), 1.85 (s, 3H, $CH_3$ thymine), 1.64–1.49 (m, 2H, CH = $CHCH_2CH_2$), 1.28–1.20 (m, 12H, $CH_2$), 1.1–0.9 (t, $J$ = 8.80, 2H, $SiCH_2$), 0.02 (s, 9H, $Si(CH_3)_3$). $^{13}$C NMR (75 MHz), $\delta$ (ppm): 284.2, 232.3, 227.6, 217.4, 170.5, 167.0, 163.9, 156.5, 150.6, 143.6, 141.1, 140.9, 140.6, 130.0, 127.6, 126.9, 122.8, 119.8, 110.3, 66.7, 65.4, 64.2, 60.7, 57.4, 52.5, 47.9, 44.1, 47.1, 38.5, 39.7, 33.5, 30.0–28.0, 21.2, 17.2, 12.2. FT-IR (neat): $\nu$ = 2000.9 (CO), 1879.0, 1840.6 (CO), 1712.7, 1679.1 (CO) $cm^{-1}$. MS (FAB$^+$): 1035 [$M$ + H–CO]$^+$, 1007 [$M$ + H–2CO]$^+$, 951 [$M$ + H–4CO]$^+$. Anal. Calcd. for $C_{53}H_{70}CrN_6O_{12}Si$: C = 59.87%, H = 6.64%, N = 7.90%, found C = 59.21%, H = 6.68%, N = 7.57%.

*2.13. Product **12b***

Orange oil. $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ (ppm): 8.36 (bs, 1H, NH thymine), 7.87–7.80 (m, 2H, arom.), 7.77–7.68 (m, 2H, arom.), 7.02 (s, 1H, C=CH thymine), 5.62–5.44 (m, 1H, CH=C*H*), 5.35–5.28 (m, 1H, CH=C*H*), 4.88–4.80 (m, 2H, $COOCH_2$–thymine), 4.48–4.42 (m, 1H, HNC*H*COO), 4.31–4.15 (m, 4H, $COOCH_2$, Phth–$NCH_2$), 3.92–3.85 (m, 4H, $CH_2OCH_2$), 3.74–3.59 (m, 2H, thymine–$NCH_2$), 3.47–3.40 (m, 2H, $CH_2NCH_{2ax}$), 3.30–3.25 (m 2H, $CH_2NCH_{2eq}$), 2.95–2.88 (m, 4H, CH=CHC$H_2$CH, $NNCH_2$), 2.81 (s, 3H, $CH_3$ *cis*), 2.77 (s, 3H, $CH_3$ *trans*), 2.67–2.62 (m, 2H, $CH_2CH = CHCH_2CH_2$), 1.85 (s, 3H, $CH_3$ thymine), 1.26–1.24 (m, 12H, $CH_2$), 0.95 (t, $J$ = 8.6 2H, $CH_2Si$), 0.012 (s, 9H, $Si(CH_3)_3$), $^{13}$C NMR (75 MHz) $\delta$ (ppm): 277.4, 232.0, 227.6, 217.0, 172.9, 170.3, 169.1, 167.6, 150.7, 141.2, 133.8, 134.3, 131.8, 124.7, 123.5, 110.0, 64.2, 60.6, 57.6, 48.3, 47.9, 46.4, 37.8, 32.0–26.0, 21.3, 17.4, 12.3. FT-IR: 3192 (NH), 1998.9 (CO), 1921 (CO), 1873 (CO), 1836 (CO), 1774, 1717, 1679 $cm^{-1}$. MS (FAB$^+$): 970 [$M$]$^+$, 942 [$M$ − CO]$^+$, 807 [$M$ + H–$Cr(CO)_4$]$^+$. Anal. Calcd. for $C_{46}H_{62}CrN_6O_{12}Si$: C = 56.89%, H = 6.44%, N = 8.65%, found C = 56.65%, H = 6.68%, N = 8.57%.

*2.14. Product **13***

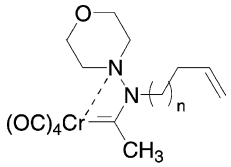
Orange oil. $^1$H NMR($CDCl_3$, 300 MHz) $\delta$ (ppm): 5.40–5.36 (m, 2H, C*H*=C*H*), 3.99–3.88 (m, 8H, $CH_2OCH_2$), 3.64–3.58 (m, 4H, $NNCH_2$ *cis*), 3.50–3.44 (m, 4H, $NNCH_2$ *trans*), 3.11–3.04 (m, 4H, $NCH_{2ax}$), 2.96–2.84 (m, 4H, $NCH_{2eq}$), 2.81 (s, 3H, $CH_3$ *cis*), 2.72 (s, 3H, $CH_3$ *trans*), 2.01–1.95 (m, 2H, C$H_2$CH=CHC$H_2$ *cis*), 1.66–1.54 (m, 2H, C$H_2$CH=CHC$H_2$ *trans*), 1.44–1.34 (m, 28H, $CH_2$); $^{13}$C ($CDCl_3$, 75 MHz) $\delta$ (ppm): 287.8, 231.8, 228.5, 217.4, 130.2, 129.0, 64.3, 57.6, 44.1, 38.4, 32.0–26.0. FT-IR: $\nu$ = 2000 (CO), 1966.7 (CO), 1922 (CO) $cm^{-1}$. MS (FAB$^+$): 865 [$M$]$^+$, 775 [$M^-$ − 3CO]$^+$, 721 [$M$ − 5CO]$^+$, 666 [$M$ − $Cr(CO)_4$–CO]$^+$, 636 [$M$ − 8CO]$^+$, 584 [$M$ − 8CO–Cr]$^+$. Anal. Calcd. for $C_{40}H_{60}Cr_2N_4O_{10}$: C = 55.80%, H = 7.02%, N = 6.51%, found C = 55.96%, H = 7.15%, N = 6.78%.

*2.15. IR analysis*

*2.15.1. Sample preparation*

Organic solutions: a stock solution ($1 \times 10^{-3}$ M) of carbene **12b** was prepared in ethyl acetate. Successive dilutions ($1 \times 10^{-5}$ to $1 \times 10^{-7}$ M) were performed

with ethyl acetate. Thirty-microliter aliquots were withdrawn by means of a Pipetman™ (Gilson) and transferred immediately to 1.5 ml microtubes (Eppendorf). The solutions were quickly evaporated to dryness on a Speedvac concentrator (Savant Inc.). Samples suitable for IR analysis were obtained by dissolving the residue in 30 μl of carbon tetrachloride and injected in the cell with a micro-syringe (Hamilton).



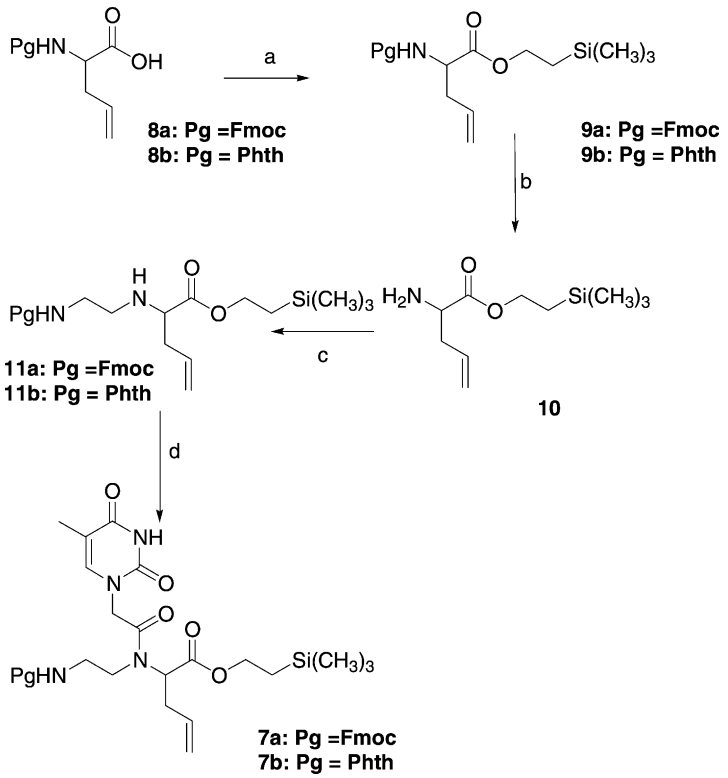

**6a: n = 3**
**6b: n = 8**

Fig. 2. Tetracarbonyl hydrazino Fischer-type carbene complexes.

*2.15.2. Sample preparation*

Aqueous solutions: a stock solution ($1 \times 10^{-3}$ M) of carbene was prepared in isopropanol. Successive dilutions ($5 \times 10^{-4}$ to $5 \times 10^{-5}$ M) were performed with phosphate buffer pH 7.4 containing 0.15 M NaCl. Five-microliter aliquots of the solutions were spotted on 6 mm diameter discs punched in 7 cm × 10 cm nitrocellulose sheets (HAHY, Millipore). The solvent was let to dry in the air for at least 30 min before IR analysis.

The IR spectra were recorded on a MB100 FT-spectrometer (Bomem). Solutions were analysed in an ultra-low volume gold light-pipe cell [17] placed in the microbeam side compartment of the spectrometer equipped with a liquid nitrogen cooled InSb detector. Nitrocellulose discs were analysed in the main compartment of the spectrometer equipped with a liquid nitrogen cooled MCT detector. The IR spectral data (1800–2200 $cm^{-1}$ range) were manipulated on a PC-compatible microcomputer using the Bomem Easy



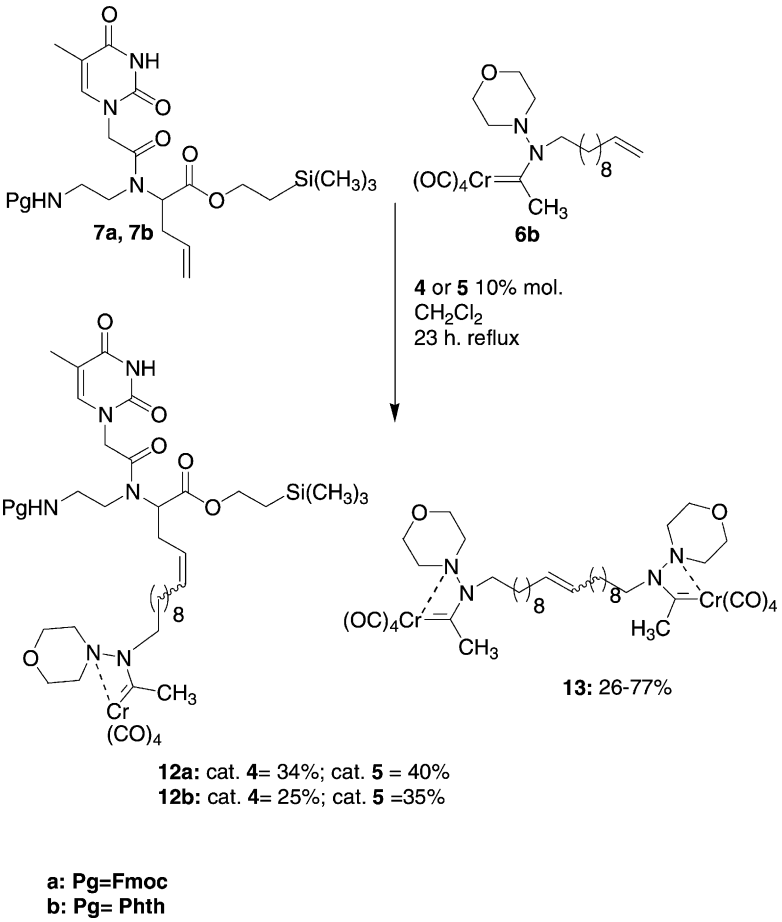

Scheme 2. Synthesis of PNA monomers **7a** and **7b**. (a) $HO(CH_2)_2Si(CH_3)_3$, DCC, DMAP, AcOEt; (b) piperidine, $CH_2Cl_2$, or hydrazine monohydrate in MeOH; (c) $PgNHCH_2CHO$, MeOH, $ZnCl_2$, $NaCNBH_3$; (d) thymine–1- acetic acid, DHBtOH, DCC, DMF.

software. Forty-three co-added interferograms (collection time 1 min) were apodised using cosine function and then Fourier-transformed to yield 4 $cm^{-1}$ resolution spectra. The IR spectrum of $CCl_4$ (organic solutions samples) or of untreated disc (aqueous solution samples) was recorded at the end of each series of measurements and used for spectral subtraction of the water vapour or the nitrocellulose absorptions, respectively. The 1924 $cm^{-1}$ peak heights and areas were measured with the quantitative analysis function of the software and were used to construct Beer's law plots. The limit of detection was defined as the concentration (quantity) of carbene corresponding to three times the standard deviation $\sigma_y$ of the measured signal.

## 3. Results

We and other authors have recently studied the ring closing and cross-metathesis reactions of appropriately designed Fischer-type carbene complexes of chromium, and we have demonstrated the compatibility of these substrates with the Grubbs catalysts **4** and **5** utilised for such reactions [18,19].

In particular, we utilised the tetracarbonyl hydrazino carbene complexes of chromium of general structure **6** (Fig. 2), whose synthesis and reactivity have been set up and studied in our laboratory [12,13,20,21]. These complexes are very stable and exhibit peculiar structural and reactivity characteristics, as a consequence of the chelation of the nitrogen atom to the chromium




Scheme 3. Metathesis reactions.

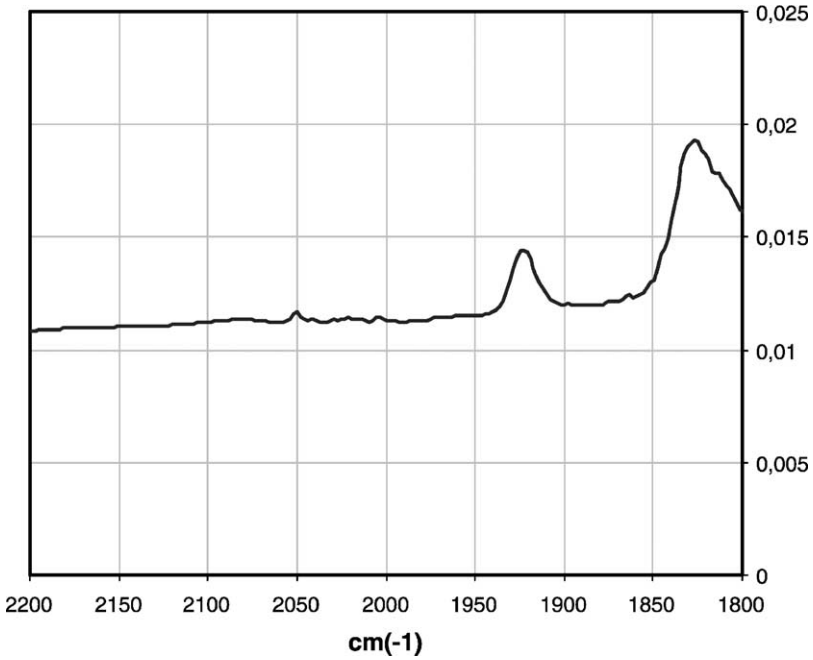



Fig. 3. IR spectrum of **12b** in $CCl_4$ solution ($5 \times 10^{-7}$ M).

atom. For example, the alkenyl chain on the nitrogen atom is in the appropriate conformation both for the ring closing and cross-metathesis reactions. During this study we also noticed that, in order to obtain reasonable yield in the cross-metathesis product, at least five carbon atoms were needed in the unsaturated chain present on the chromium carbene complex [19].

The new protected PNA monomers **7a** and **7b**, necessary for the synthesis of the target labelled PNA monomer **3**, were synthesised as shown in Scheme 2. Compounds **8a** and **8b** were synthesised as previously reported [22–24]. They were then protected by esterification with trimethylsilyl ethanol to afford **9a** and **9b**. The deprotection of the amino group with piperidine or with hydrazine monohydrate gave the amino ester **10** which was then submitted to reductive amination with 9-fluorenylmethoxycarbonyl or phthaloyl *N*-protected aminoacetaldheyde affording **11a** and **11b**. Thymine–1-acetic acid was eventually coupled to the PNA backbone leading to the PNA monomers **7a** and **7b** with an overall yield of 40%. The coupling was performed using 3-hydroxy-3,4-dihydro-4-oxo-1,2,3-benzotriazine as the condensing agent, following the standard procedures reported in [25].

Table 1
Regression analysis of the calibration curves of compound **12b**

| Sampling | Parameter observed | $a$[a] | $b$[a] | $R^2$[b] | $\sigma_y$[c] | Limit of detection |
|---|---|---|---|---|---|---|
| Solution sampling | Absorbance | $-6 \times 10^{-5}$ | 4776.3 | 0.9797 | 0.000232126 | $1.58 \times 10^{-7}$ M |
| | Area | −0.0099 | 93044 | 0.9457 | 0.00743485 | $3.46 \times 10^{-7}$ M |
| Membrane sampling | Absorbance | $4 \times 10^{-5}$ | 2907.1 | 0.9801 | 0.00035051 | $3.47 \times 10^{-7}$ mmol |
| | Area | −0.0301 | 97724 | 0.9745 | 0.01333535 | $7.17 \times 10^{-7}$ mmol |

[a] Linear regression: $A = aQ + b$; $A$ is the absorbance or the area of the peak, $Q$ the quantity of compound in mmol or concentration.
[b] Determination coefficient.
[c] Standard deviation.

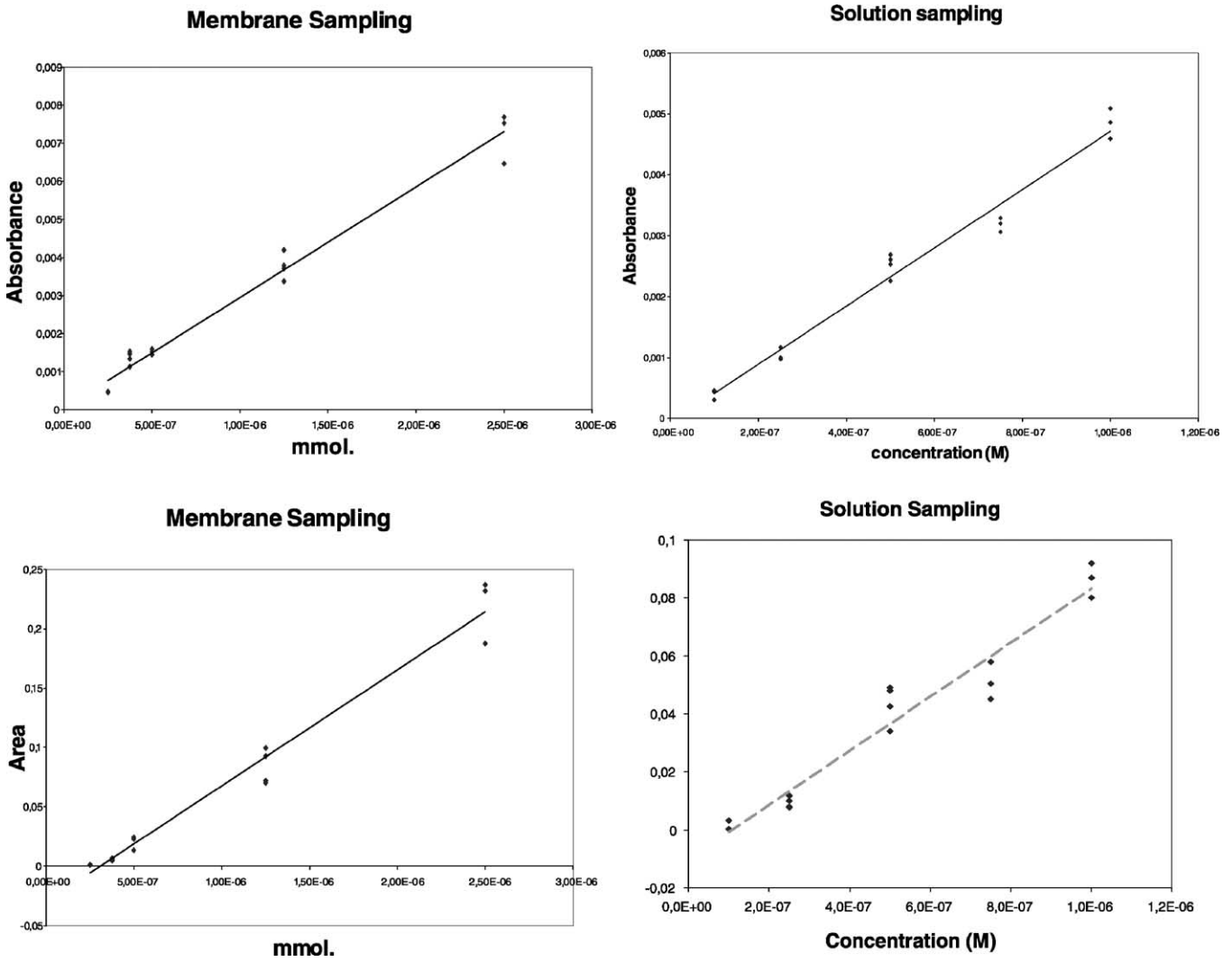



Fig. 4. Plots of the absorbance/area of the 1924 $cm^{-1}$ band vs. concentration of compound **12b**.

We realised the metathesis reactions between the PNA monomers **7a** and **7b** and Fischer-type carbenes **6a** and **6b** (Scheme 3). The reactions were run in $CH_2Cl_2$ with 10 mol% of both catalysts **4** and **5**. According to previous observations [18,19], it was not possible to obtain the desired cross-metathesis product using carbene **6a**, probably due to steric hindrance. On the contrary, performing the cross-metathesis reaction between the Fischer-type carbene **6b**, and both **7a** and **7b**, we obtained the expected products **12a** and **12b** in appreciable yields and in a very high *cis*/*trans* ratio (20:80 for **12a** and 10:90 for **12b**) (Scheme 3); in addition, the dimerization product **13** arising from **6b** was also isolated, while we did not observe any dimerization of PNA monomers **7a** and **7b**.

Compounds **12a** and **12b** are the first examples of chiral carbene complex conjugates of a PNA monomer. Since both enantiomers of allylglycine are commercially available, compounds **12** in principle could be synthesised in the enantiomerically pure form.

In order to explore the possibility of using the carbonyl ligands stretching frequencies of the complexes **12a** and **12b** as spectroscopic probes, we recorded and analysed the FT-IR spectrum of bioconjugate **12b**. As mentioned earlier, Fischer-type carbenes have, indeed, very characteristic intense IR absorption bands in the 2100–1800 $cm^{-1}$ region. We investigated the minimum tracer quantity detectable by IR spectroscopy both in solution and on nitro-cellulose membranes. We chose these techniques because they have different peculiarities. The solution sampling in chlorinated solvent allowed us to reach lower concentration comparing with the membrane sampling which is less sensitive, but allowed us to study physiological solutions.

Product **12b** has a *Cs* symmetry, then it presents four active vibration mode in IR spectroscopy. It is possible to observe them while recording nujol or neat spectra, but only the band at 1924 $cm^{-1}$ could be detected working at low concentration (Fig. 3). The 1924 $cm^{-1}$ band was observable in the IR spectrum until the concentration of $1 \times 10^{-7}$ M (3 pmol) for the solution sampling and until 250 pmol for the membrane sampling. Plots of the absorbance/area of the 1924 $cm^{-1}$ band versus concentration of compound **12b** are shown in Fig. 4. A regression analysis was successfully applied to the plots (Table 1) indicating the validity of Beer's law in the range of concentrations considered. We observed in both cases that analysis using the absorbance values afforded better calibration curves. It was possible to calculate a theoretical limit of detection from the lines parameters. Results are shown in Table 1. With both techniques we found that absorbance measurements gave numeric values of limit of detection closer to those obtained from the experimental data. This is probably due to the better determination coefficient ($R^2$) found for absorbance/concentration plots as compared to area plots.

## 4. Conclusions

In conclusion, in this preliminary study, we have synthesised two differently protected derivatives of a new chiral PNA monomer (compounds **7a** and **7b)** and their Fischer-type carbene conjugates (compounds **12a** and **12b**) obtained by metathesis reaction with Grubbs catalysts **4** and **5**. Complex **12b** could be detected by FT-IR spectroscopy in solution until a concentration of $1 \times 10^{-7}$ M (3 pmol) and until 250 pmol for membrane sampling. This work is still in progress, our aim is to synthesise oligomeric PNAs in which the organometallic moiety is included in a traditional PNA chain. The possibility of functionalising the α-carbon of the glycine unit in order to attach different organometallic moieties to the PNA backbone will enable us to achieve a wide range of bio-organometallic conjugates with different and improved spectroscopic or electrochemical properties. Quantity of carbene corresponding to three times the standard deviation $\sigma_y$ of the measured signal.

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