UNIVERSITA' DEGLI STUDI DI MILANO
Dipartimento di Chimica Organica e Industriale
Via Golgi 19, 20133 Milan (Italy)
stefano.maiorana@unimi.it

Peptide Nucleic Acids: a Challenge at the Beginning of the Post-Genome Era

Stefano Maiorana

IASOC 2004

Expression of gene-stored information

GENE = a single unit of genetic information

DNA —TRANSCRIPTION→ RNA —TRANSLATION→ PROTEIN

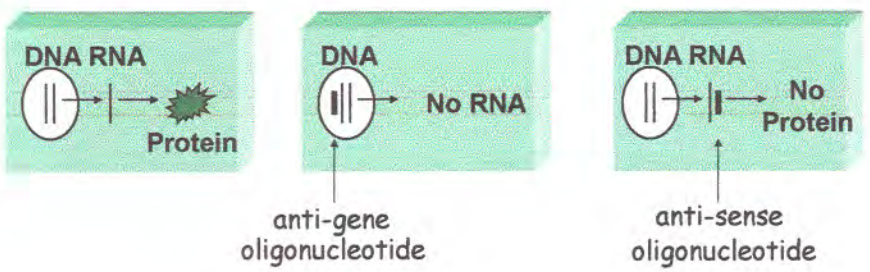
A single mistake in the sequence of nucleobases (Single Nucleotide Polymorphism, SNP) can determine severe diseases and different response to drugs.

IASOC 2004

Pharmaceutical science + knowledge of genes and proteins

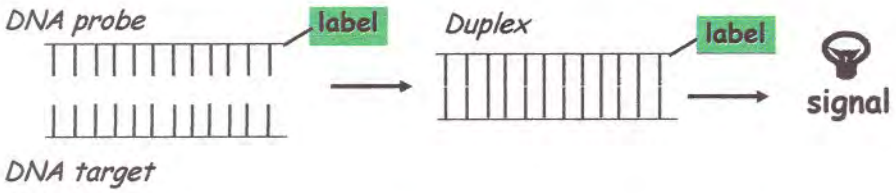
⇩

pharmacogenomics



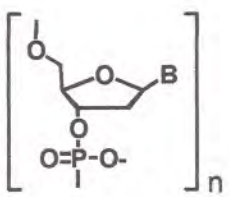
IASOC 2004

DNA detection is based on the fact that DNA exists as a duplex of complementary sequences. Thus, a single stranded DNA is able to pick up its complementary target specifically from a mixture of genes. If a probe DNA is labelled appropriately, the duplex formed is detected easily and with sensitivity.

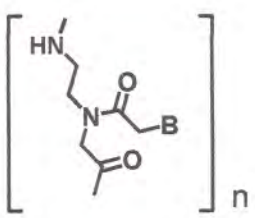


Stability and sequence specificity as well as binding affinity of natural DNA are often worst than desired. Therefore much effort has been directed to the development of DNA analogues with improved properties.

PEPTIDE NUCLEIC ACIDS (PNAs): a major breakthrough



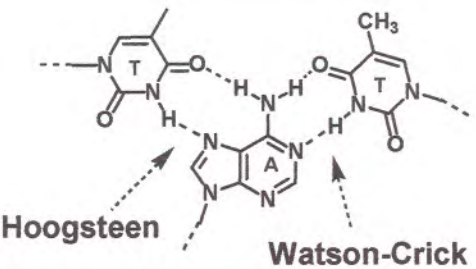
DNA



PNA:

high bio-stability, high affinity and sequence specificity for DNA

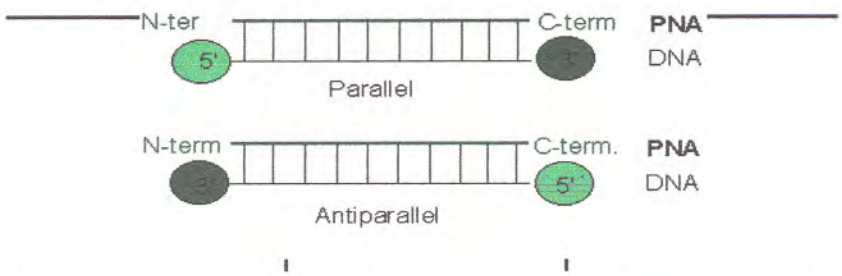
P.E. Nielsen, *Science*, 1991, *254*, 1497.



DUPLEX PNA / DNA

TRIPLEX $(PNA)_2$/DNA

IASOC 2004



Melting temperatures of duplexes formed by PNA and DNA 15-mer TGTACGTCACAACTA.

DUPLEX	Tm (antiparallel)/°C	Tm (parallel)/°C
PNA:DNA	69.5	56.1
PNA:RNA	72.3	51.2
DNA:DNA	53.3	-
DNA:RNA	50.6	-

IASOC 2004

PEPTIDE NUCLEIC ACIDS

Potential applications in:

- pharmacogenomics
  - *anti-gene and antisense drugs*
- diagnostics

IASOC 2004

PEPTIDE NUCLEIC ACIDS

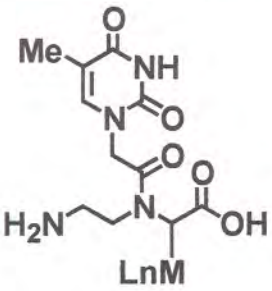
Drawbacks:

- Low solubility in physiological medium
- Low lipophilicity
- Difficult cell up-take
- Sensitivity to some hydrolases
- Lack of relevant analytical probes within their backbone

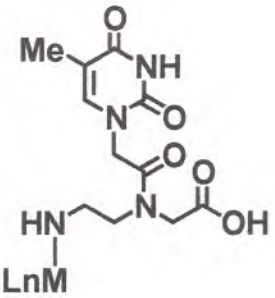
modified PNA

IASOC 2004

Metal-PNA conjugates



M = Cr, W, Fe

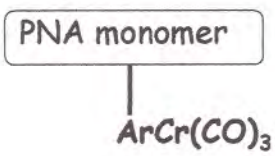


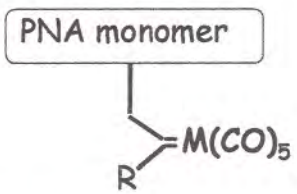
LnM = -ArCr(CO)$_3$, Fischer-type carbene, Ferrocene

AIMS ⇄ 1. lipophilic PNAs
2. analytical probes

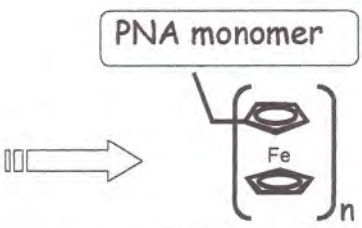
IASOC 2004

.....Our work

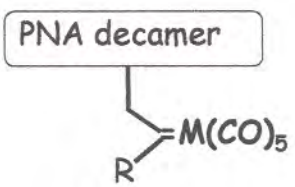




Maiorana, S., Licandro E., Zinzalla G., Giannini C. *Synlett*, . 2004, 1044.
Licandro E., Maiorana S., Baldoli C., Vandoni, B. , Salmain, M. *J. Mol. Catalysis*, 2003, *204*. 165.
Baldoli C., Maiorana, S., Licandro E., Zinzalla G., Perdicchia, D. *Org. Lett.* 2002, *4*, 4341.



n = 1, 2, 3



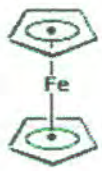
M = Cr, W

IASOC 2004

Ferrocene as the analytical probe

Ruthenium, osmium, iron, rhodium, and copper complexes have so far been proposed for the electrochemical detection of DNA

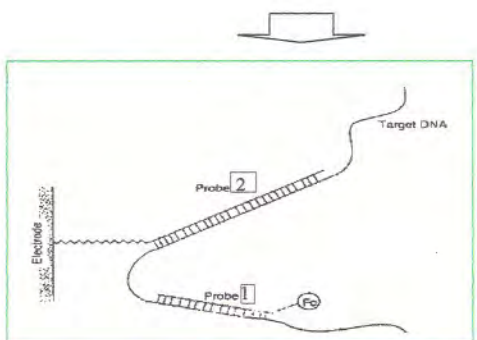
Among them, ferrocene is the most convenient



- very stable
- undergoes reversible one-electron oxidation
- exhibits characteristic UV-Vis absorptions that facilitate its detection e.g. during HPLC purification

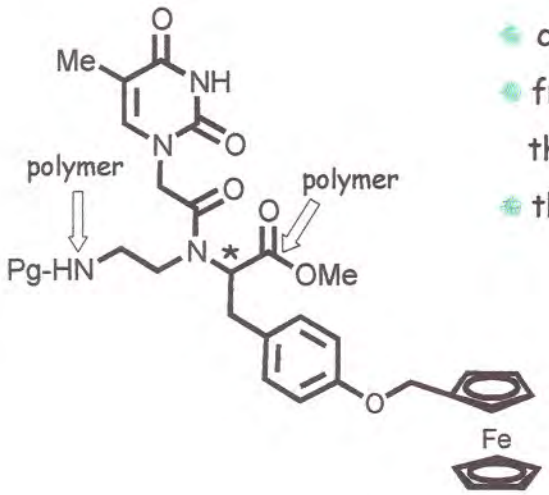
Gene sensor using ferrocenyl oligonucleotide

T. Ihara et al.; *Chem Commun*, **1997**, 1609



Target DNA binds two different probe DNAs. Probe 1 labelled with the Fc unit and then probe 2 which is immobilized on the electrode

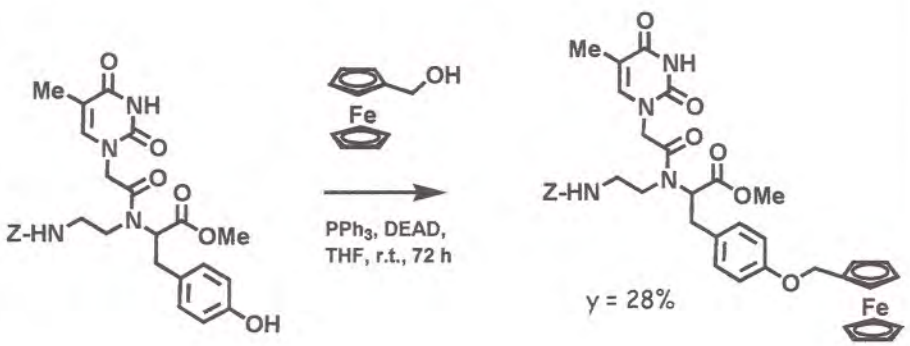
Mono-ferrocene-unit PNA monomer



- chiral
- free H bond sites in the nucleobase
- thymine as a base

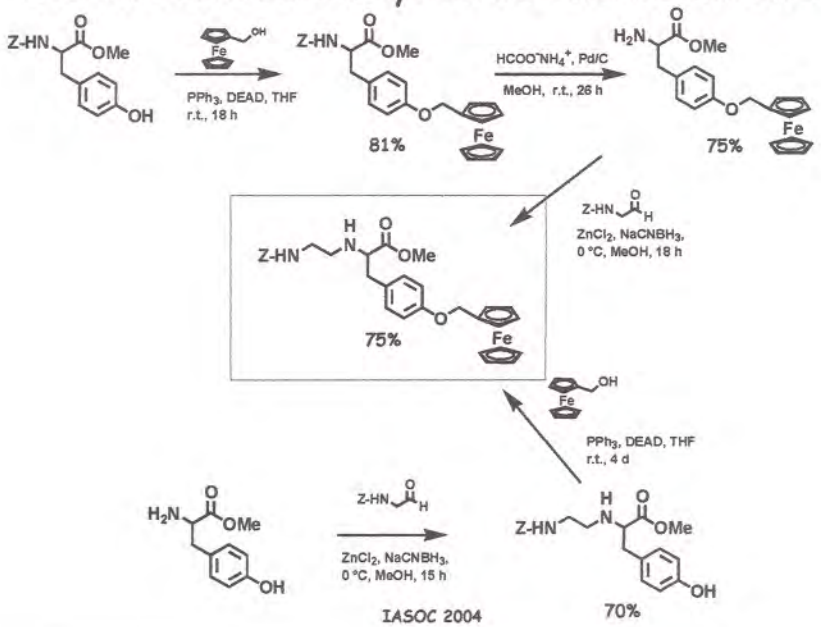
IASOC 2004

The Mitsunobu on the PNA monomer



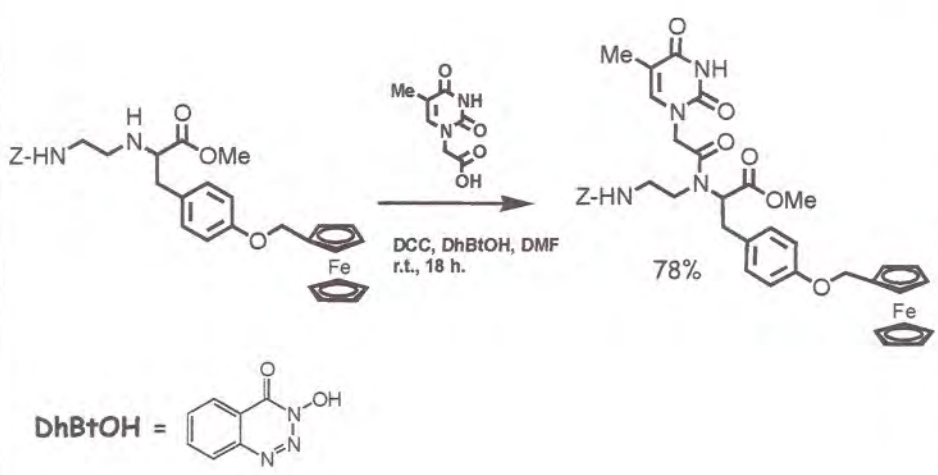
IASOC 2004

The Mitsunobu on tyrosine and backbone



IASOC 2004

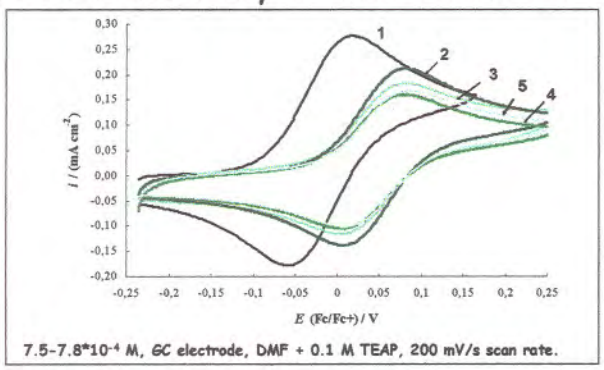
The final step: Fc-labelled PNA monomer



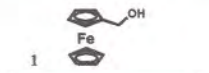
DhBtOH =

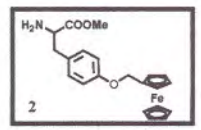
IASOC 2004

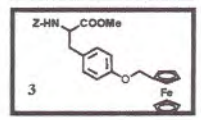
Cyclovoltammetry of ferrocene dervs

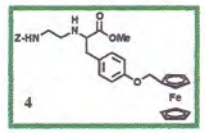


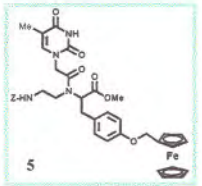
7.5-7.8*10^{-4} M, GC electrode, DMF + 0.1 M TEAP, 200 mV/s scan rate.











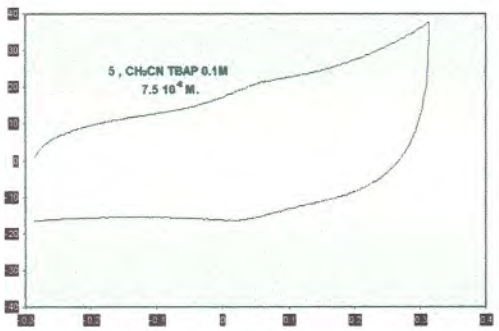
- single and quite reversible monoelectronic wave
- shift of all potentials in the positive direction with respect to ferrocene methanol

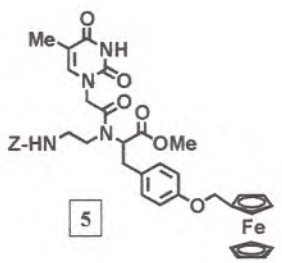
⇩

- increased stability to the oxidation
- the potential shift remains almost unchanged even increasing the molecular complexity

IASOC 2004

Analysis of limiting detection

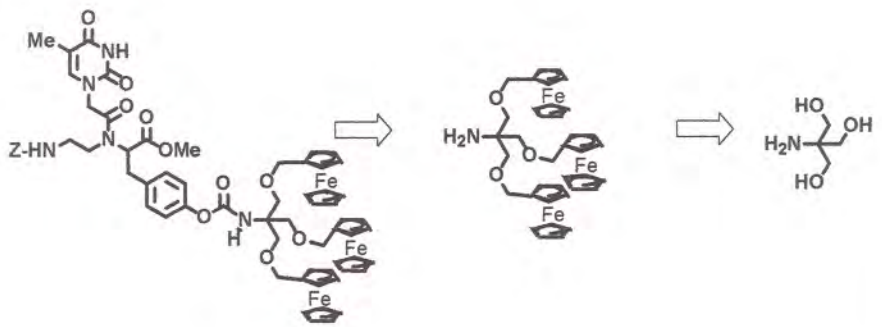




CV obtained on GC electrode (r = 2 mm) , 200 mV s^{-1} scan rate, 298.15 K

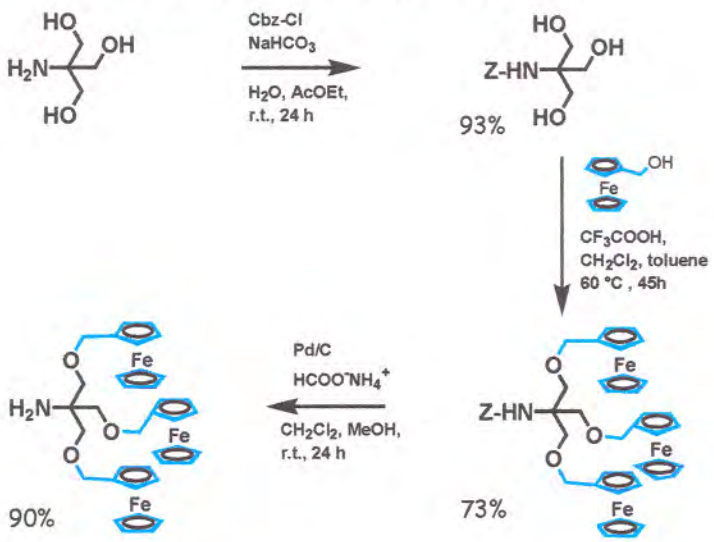
IASOC 2004

New multifunctional organometallic PNA monomer



IASOC 2004

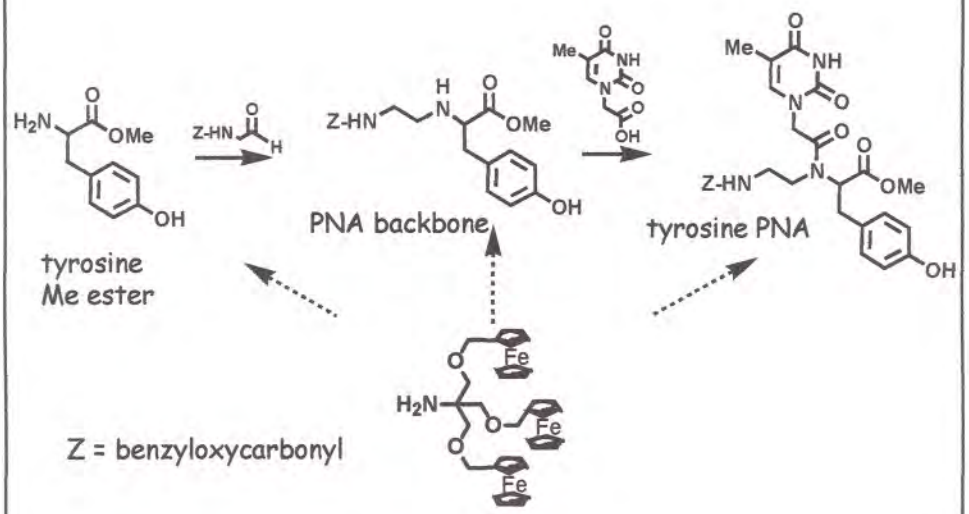
Tris ferrocene derivatives



It Patent N. MI2004A001427(2004)

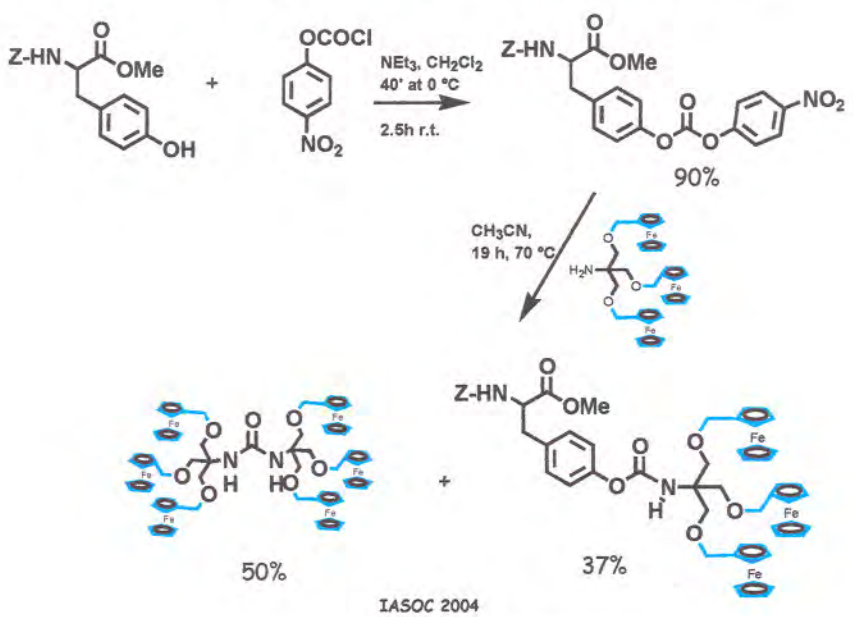
IASOC 2004

Three different possibilities



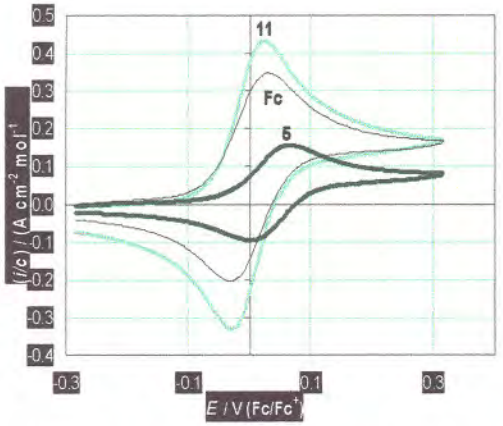
IASOC 2004

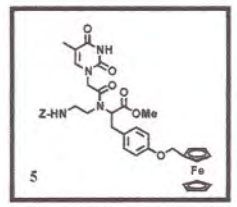
Synthesis of tris-ferrocene tyrosine

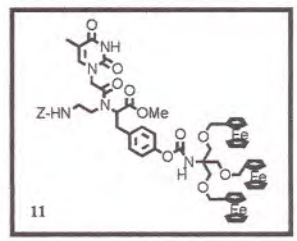


IASOC 2004

Effect of adding more ferrocene groups in the PNA monomer





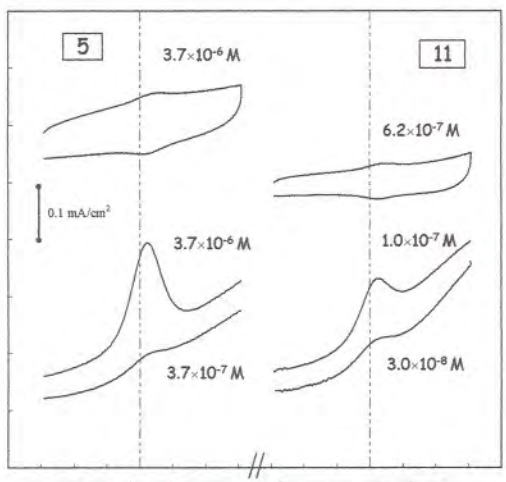


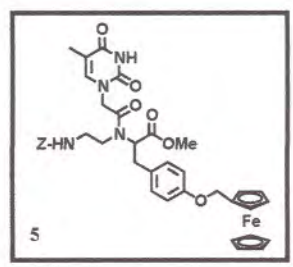
7.5-7.8×10^{-4} M, GC electrode, CH_2Cl_2 + 0.1 M TBAP, 20 mV/s scan rate.

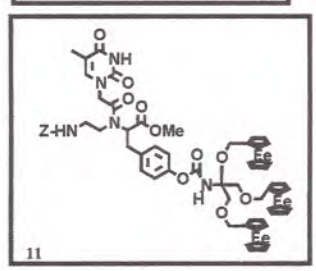
With three ferrocene groups the current density ratio is 2.8:1.
Current density ÷ to number of Fc units.

IASOC 2004

Analysis of limiting detection



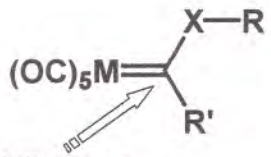




Voltammetric patterns obtained at 298 K on GC disk electrode (diameter = 3 mm) working with extremely diluted solutions of PNA monomers **5** *and* **11** *in ACN + 0.1 TBAP medium. CV: 0.2 V s^{-1} scan rate, DPV: 0.05 V s^{-1}, modulation time 2 ms, modulation amplitude 25 mV, step potential 5 mV, interval time 0.1 s*

IASOC 2004

Fischer-type carbene complexes



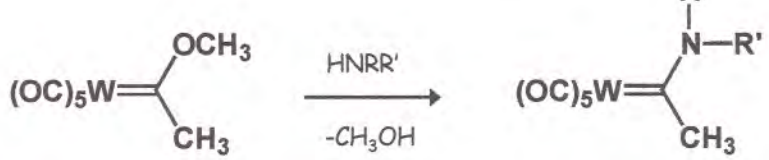
Electrophilic center

M = Cr, Mo, W

X = NR", O, S

R, R' = alkyl, aryl etc..

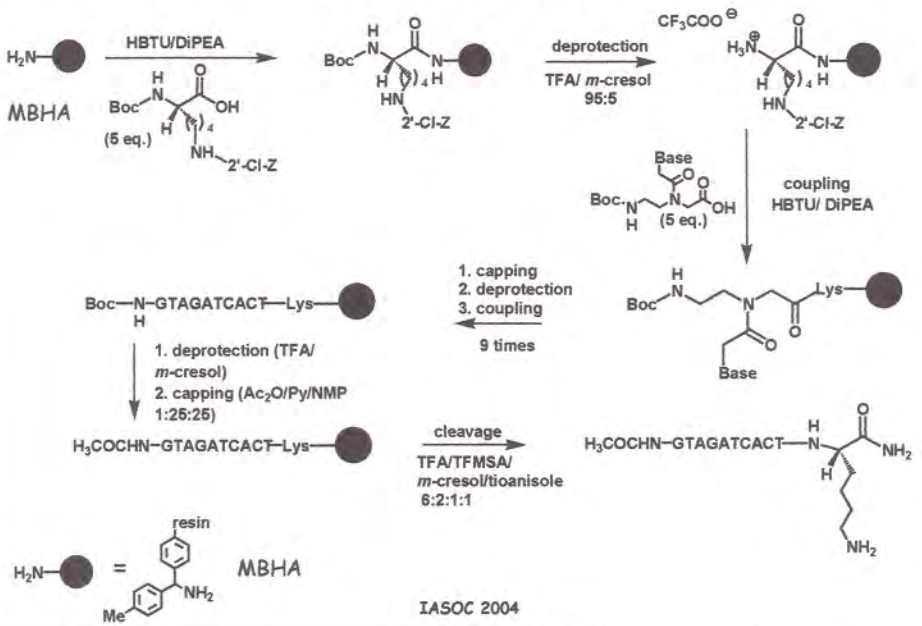
Aminolysis reaction



Tungsten alkoxy carbene *amino carbene*

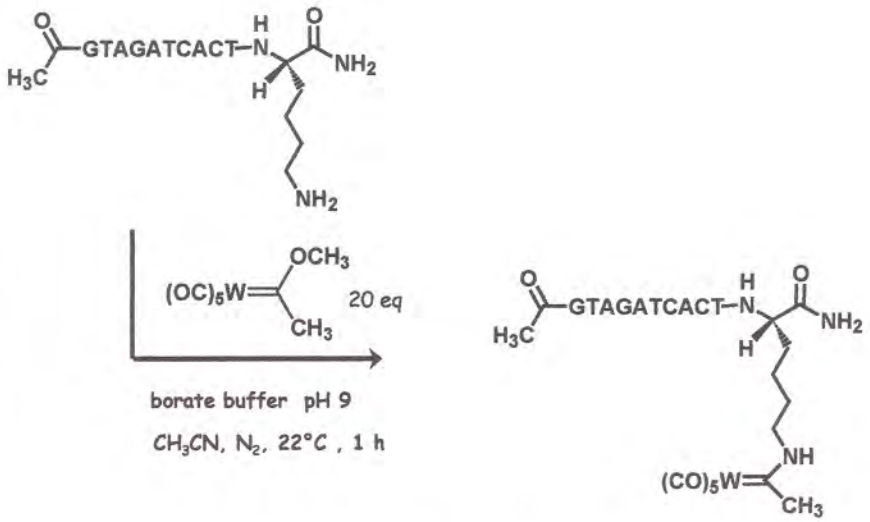
IASOC 2004

SPS of PNA decamer: GTAGATCACT



IASOC 2004

Aminolysis of Fischer-type carbene



IASOC 2004

Recent reviews

1. Uhlmann, E.; Peyman, A.; Breiphol, G.; Will, D. W. **PNA: Synthetic Polyamide Nucleic Acids with Unusual Binding Properties**. *Angew. Chem. Int. Ed.* (1998), 37, 2792-2823
2. Nielsen P E **Peptide nucleic acids: on the road to new gene therapeutic drugs.** Pharmacology & toxicology (2000 Jan), 86(1), 3-7.
3. Ray A; Norden B **Peptide nucleic acid (PNA): its medical and biotechnical applications and promise for the future.** *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* (2000 Jun), 14(9), 1041-60.
4. Ray, Arghya; Norden, Bengt. **Peptide nucleic acid (PNA): its medical and biotechnical applications and promise for the future.** *FASEB Journal* (2000), 14(9), 1041-1060.
5. Ganesh, Krishna N.; Nielsen, Peter E. **Peptide nucleic acids: analogs and derivatives.** *Current Organic Chemistry* (2000), 4(9), 931-943.
6. Koppelhus, Uffe; Nielsen, Peter E. **Cellular delivery of peptide nucleic acid (PNA).** *Advanced Drug Delivery Reviews* (2003), 55(2), 267-280.
7. Kaihatsu, Kunihiro; Janowski, Bethany A.; Corey, David R. **Recognition of chromosomal DNA by PNAs.** Chemistry & Biology (2004), 11(6), 749-758.
8. Nielsen, P. E. **Peptide Nucleic Acids: Protocols and Applications, Second Edition**,(2004) Horizon Bioscience, Wymondham, UK.

IASOC 2004